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EXAMINER	
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BEFORE THE BOARD OF PATENT APPEALS

AND INTERFERENCES

Paper No. 14

Application Number: 09/546,143
Filing Date: April 10, 2000
Appellant(s): MATZINGER ET AL.

Aaron F. Dubberley

For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 10-12-01.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims

Appellant's brief includes a statement that claims 10-27 do not stand or fall together and provides reasons as set forth in 37 CFR 1.192©(7) and ©(8).

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

5,583,222	**BARBIER et. al.**	12-1996

Adams, C.P. et. al. "Total Synthesis of balanol: a potent protein kinase C inhibitor of fungal origin" Jounral of Chemical Society, Perkin Transaction I, 1975, pp. 2355-2362.

Krogsgaard-Larsen P. et. al. "Inhibitors of GABA Uptake. Syntheses and H NMR Spectroscopic Investigations of Guvacine, (3RS, 4SR)-4-hydroxypiperidine-3-carboxylic Acid, and Related Compounds" Acta Chemica Scandinavica B, vol. 32, no. 5 (1978), pp. 327-334.

Wade, G. L. Jr. ORGANIC CHEMISTRY, © 1987, pages 103 and 115, Prentice Hall Inc., Englewood Cliffs, New Jersey (cited as a basic principle for geometric isomerism – not to be construed as a new prior art).

(10) First Ground of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 112

The following is a quotation of the **second** paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 12, 14, 15, 17, 19, 21, 23, and 25 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Said claims recite the limitation of "amino protecting group" which has no description in the specification other than "tert.-butoxycarbonyl" as a sole representative of said group. Thus, one skilled in the art cannot ascertain what other groups can be considered as an "amino protecting group". Therefore, **the metes and bounds** of the invention is indefinite.

The following is a quotation of the **first** paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 12, 14, 15, 17, 19, 21, 23, and 25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for R^4 as a tert-butyl ester, tert-

butyl carboxylate, or tert-butoxycarbonyl, does not reasonably provide enablement for the genera with R^4 as another functional group serving as an "amino protecting group". The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The disclosure does not provide guidance as to what functional groups, and/or rings can be considered as an amino protecting group. Its mere statement *"R^4 is a protecting group, preferably a tert.-butoxycarbonyl group"* is insufficient for one skilled in the art to consider what other groups can be an "amino protecting group". In considering enablement, undue experimentation is an important factor. Here, the scope of "amino protecting group" is undoubtedly broad, and the generic teaching for preparation only gears toward one "amino protecting group", namely, tert.-butoxycarbonyl. Thus, regarding other groups, one skilled in the art will have to carry out undue experimentation, as the chemical art is unpredictable. Note, the Federal Circuit has repeatedly held that **"the specification must teach those skilled in the art how to make and use the** *full scope of the claimed invention* **without 'undue experimentation'"** (see **In re Wright**, 999 F. 2d 1557, 1561, 27 U.S.P.Q. 2d 1510, 1513 (Fed. Cir. 1993)). Also, the disclosure does not provide the starting material for R^4, nor a source for an "amino protecting group", and thus, undue experimentation is inevitable for one skilled in the art to make and use compounds with R^4 as a group other than tert.-butoxycarbonyl. See **In re Howarth**, 210 U.S.P.Q 689, 693 regarding insufficient enablement. Note, in said case, the starting material was not disclosed, nor was a source named. The court, then, ruled that "**burden rests upon applicant who** *chooses to rely upon*

general knowledge in the art **to render his disclosure enabling to establish that those of ordinary skill in the art can be expected to possess or know where to obtain this knowledge;...**" Thus, 'no disclosure of starting material' is a sound reason to render a specification with insufficient enablement.

(11) Response to Argument on the above issues of 112/ 1st and 2nd paragraphs for claims 12, 14, 15, 17, 19, 21, 23, and 25

Applicant disputes the indefiniteness of the terms "amino protecting group" for the following reasons:

a. Said terms are well-known in the art;

b. Over 500 US patents recite said terms in their claims.

c. Citing textbook of Green a long with case laws such as: In re Skoll (187 USPQ 481), In re Fuetterer (138 USPQ 217), In re Bowen (181 USPQ 48), In re Robins (166 USPQ 552) to support applicant's position.

d. The definition of R^4 as an "amino protecting group" is a functional language.

e. As for enablement, applicant feels that it is not necessary to teach what is well known in the art.

Despite applicant's argument, the issues of 112/1st and 2nd paragraphs should be upheld for the following reasons:

i. The issue of indefiniteness is not whether one skilled in the art can understand a term (or terms), rather it is the metes and bounds of the invention. In the instant case, the terms "amino protecting group" as defined for R^4 does not clearly define the scope of claims 12, 14, 15, 17, 19, 21, 23, and 25. The disclosure does not describe what functional groups, ring(s) said terms encompass, except tert.-butoxycarbonyl. Therefore, when interpreting the instant claims in light of the specification, one does not have a guidance as to what is claimed (or **not** claimed) by the terms "amino protecting group". Applicant cites the textbook of Green for description of an "amino protecting group". However, **it is still unclear whether the scope of "amino protecting group" includes groups cited by Green, or goes beyond that**. While breadth is not indefiniteness, the claims must particularly point out and **distinctly define the metes and bounds** of the subject matter that will **be protected by the patent grant.**

ii. It is recognized that the terms "amino protecting group" are recited in a lot of US patents. However, usually, said terms are used specifically in a reaction step in a claimed process. When read in light of a process, said terms have a more definite metes and bounds. In the instant case, applicant claims intermediates (i.e., compounds) having "amino protecting group" as a substituent. Because in the specification, there is no description as to what constitutes said group (other than tert.-butoxycarbonyl), the final structure of a claimed intermediate is indeterminate. Therefore, the scope of claims reciting said terms is indefinite.

iii. Case laws cited by applicant are outdated. The most recent case law is (**Genetech Inc. v. Novo Nordisk**, 108 F.3d 1361, 42 USPQ 2d 1001 (Fed. Cir. 1997)), in which the court ruled that relying on the knowledge of one skilled in the art cannot cure the deficiency in enablement. Just because a term is well-known in the art, it does not mean one skilled in the art can prepare any intermediate having any "amino protecting group". Based on what disclosed by applicant, one skilled in the art can only make compounds with R^4 as tert.-butoxycarbonyl. It is true applicant does not have to provide enablement for known processes in the art, but applicant is obligated to provide **enablement in commensurate with the scope of the claims**. In the instant case, because the terms "amino protecting group" have such a broad scope that the enablement for only tert.-butoxycarbonyl does **not sufficiently** guide the skilled chemist in preparing intermediates with other amino protecting groups.

(12) Second Ground of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371© of this title before the invention thereof by the applicant for patent.

The rejection of claim 23 under 35 U.S.C. 102(a) based on **Lamp et. al.** is withdrawn herein in light of applicant's argument.

102(b) – Claims 17, 23, and 25 are rejected under 35 U.S.C. 102(b) as being anticipated by the following references:

a. **Krogsgaard-Larsen et. al.** (Acta Chem. Scan. B, 32 (1978), pp. 327-334): Compounds 12 and 13 on page 328 are embraced by formula VIII in claim 17 with R^3 as lower alkyl, and R^4as an amino protecting group.

b. **Adams et. al.** (J. Chem. Soc. Perkin Trans. I, 1995, pp. 2355-2362): Formula X in claim 23 inherently embraces compound 20 on page 2356. Formula XI in claim 25 inherently embraces compound 21 on page 2357.

102(e) – Claim 25 is rejected under 35 U.S.C. 102(e) as being anticipated by **Barbier et. al.** (US 5,583,222). Formula XI in claim 25 inherently embraces compounds B1-B23 listed on columns 18-21.

(13) Response to Argument on 102(b), and (e) rejections for claims 17, 23, and 25

Applicant disputes that the 102 rejections are improper because said references do not disclose the cis-form of the claimed formulae. Applicant argues that the trans-form is structurally different from the cis-form, and thus, cannot anticipate the cis-form of the claimed formulae.

Applicant's argument is well taken; however, the 102 rejections based on inherency should be upheld for the following reasons:

i. Despite applicant's assertion, cis- and trans- forms of a compound have **similar structures** due to their common cores, and substituents. They only differ in the spatial orientation of their substituents. However, spatial orientation of a compound can flip flop from one form to the other because **bonds are not static**. Anyone skilled in the art would know that if a trans-form of a compound, a cis-form also **exists inevitably**. This is the most fundamental principle in stereo-chemistry. So, if a reference discloses a trans-form, then a cis-form will be inherently embraced.

ii. Regarding claim 17, compound #12 of Krogsgaard-Larsen clearly a **cis**-compound because both the –OH locates on the same side with $-C(O)OCH_3$ just as the claimed formula VIII. It is not a racemic mixture of cis- and trans- as suggested by applicant. The symbol, "(±)", refers to optical isomers of the **cis**-compound. Note, both the –OH and the $-C(O)OCH_3$ can be pointed upward or downward, and still have cis-

configuration. See Wade's ORGANIC CHEMISTRY, pages 103 and 115, as evidence for the basic principle of cis- and trans- configurations (or geometric isomerism).

(14) Third Ground of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 25 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Barbier et. al.** (US 5,583,222). On column 9, Barbier et. al. disclose a group of intermediates represented by formula III which resembles the claimed formula XI since the reference's variable Y can be taken as –NH while the –CO-A corresponds to the instant variable R^1, and R^{16} (represents an "amino protecting group") corresponds to the instant R^4. Furthermore, column 18 lists intermediates B1-B23 which have tert-butyl carboxylate as an amino protecting group (e.g., see intermediate B4). While Barbier et. al. do not disclose the cis-configuration of compounds of formula III or its species, such form is suggested in the racemic mixture of cis- and trans- represented by formula III.

Furthermore, Barbier et. al. recognizes that substituents on the heterocyclic ring can have cis-configuration as well (see column 4, line 30). Therefore, one of the ordinary skill in the art would have been motivated to make the cis-configuration of compounds of formula III because such a configuration had been acknowledged by Barbier et. al. as an alternative to trans-configuration. Thus, at the time of the invention, it would have been obvious to one skilled in the art to make intermediates of formula XI and its species because one skilled in the art can always resolve a racemic mixture of formula III by conventional methods to obtain the cis-form of formula XI claimed herein.

(15) Response to Argument on the 103 rejection for claims 25 and 26

Applicant's argues that the broad genus of the disclosed formula III does not allow one skilled in the art to select the intermediates claimed herein.

Applicant's argument cannot overcome the rejection for the following reasons:

i. Barbier et. al. not only disclose the genus of formula III, but also its species (B1-B23). So, one cannot dispute the teaching of Barbier et. al. as merely being broad.

ii. Because the disclosed species B1-B23 are in trans-form, formula III was cited in the rejection as a racemic mixture, which suggests that a cis-form is also implicitly suggested. Therefore, it is within the level of one skilled in the art to obtain the claimed cis-form from the teaching of Barbier et. al., and conventional methods of resolving cis- and trans- forms.

For the above reasons, it is believed that all rejections should be sustained.

Respectfully submitted,

Tamthom N. Truong

March 27, 2003

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Total synthesis of balanol: a potent protein kinase C inhibitor of fungal origin

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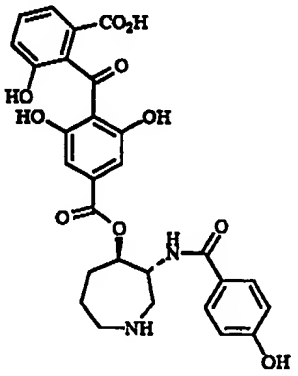
The total synthesis of the fungal metabolite balanol, a potent inhibitor of protein kinase C, is described. The synthesis includes a novel synthesis of 3-amino-4-hydroxyazepanes *via* a directed ring expansion of 3-bromopiperidin-4-ones.

Balanol **1** is a structurally novel inhibitor of protein kinase C, a family of phospholipid dependent serine/threonine protein kinases which play an important role in cell growth, signal transduction and differentiation.[1] The activated enzyme[2] has been implicated in many diseases, such as cancer, inflammation and HIV infection; therefore inhibitors of protein kinase C may be therapeutic.[3] Balanol **1** was initially isolated by workers at Sphinx Pharmaceuticals from *Verticillium balanoides*[4,5] and more recently from species of *Fusarium*[6] by a team at Nippon Roche. Synthetic interest in balanol is intense, owing to its chemical structure, biological activity and its low availability from natural sources. As our synthetic route was nearing completion, the total synthesis of balanol was recently reported by workers at Sphinx,[7] and by the Nicolaou group.[8] Here we describe a new synthesis of balanol with novel routes to the hexahydroazepine (azepane) and benzophenone portions.



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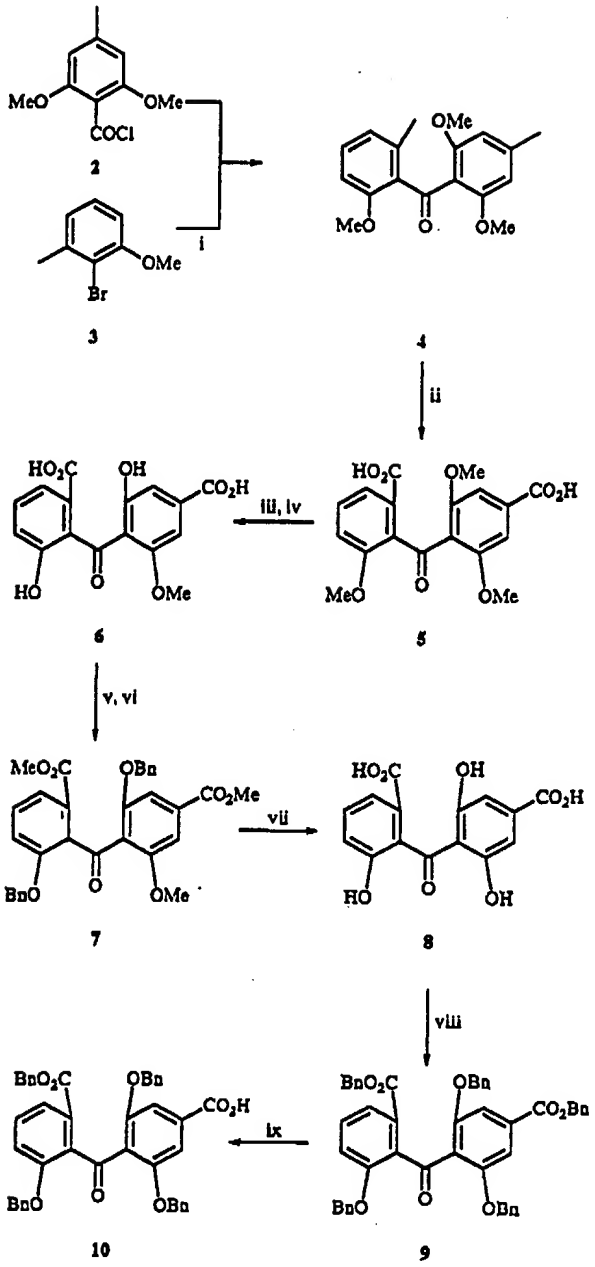
Results and discussion

The key disconnection for the synthesis of balanol is the ester bond joining the azepane and the benzoic acid. This implies that preparation of suitably protected benzophenone and azepane portions, which would be coupled together to form the ester bond, could then be deprotected to give balanol **1**.

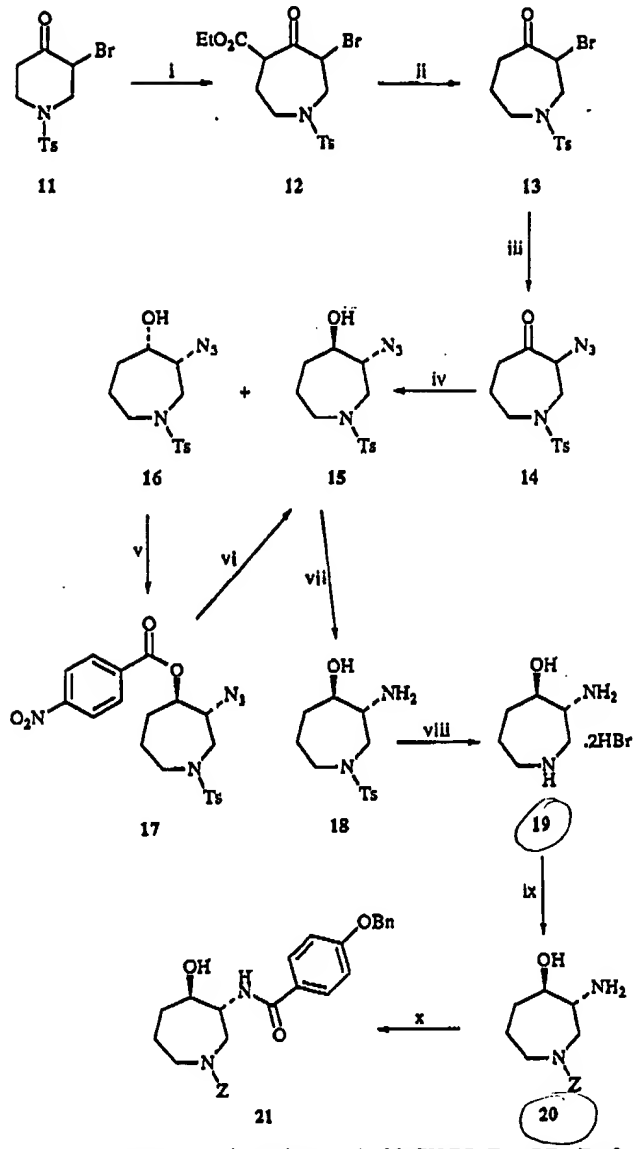
The synthesis of the benzophenone portion started from 2,6-dimethoxy-4-methylbenzoyl chloride[9] **2** and 2-bromo-3-methoxytoluene[10] **3** which were readily prepared from commercially available 3,5-dimethoxytoluene and 2-amino-3-methoxytoluene respectively, in 86 and 85% yield. Formation of the Grignard reagent of **3** and reaction of this with **2** gave the hindered benzophenone **4** in 80% yield. Oxidation of the methyl groups of **4** using potassium permanganate gave the diacid **5** in 38% yield. The diacid **5** had limited solubility and was quantitatively converted into the dimethyl ester. Treatment of this dimethyl ester with boron tribromide gave **6** in 95% yield with only small amounts of **8** being detected. Further demethylation of **6** to give the triphenolic diacid **8** directly was problematic and treatment of **6** with boron tribromide and a host of other demethylating agents yielded only traces of **8**. Possibly once **6** has been formed, its insolubility and ability to complex with reagents hinders the final demethylation. This problem was overcome by re-esterification of **6** to give the dimethyl ester followed by benzylation of the phenolic groups to give **7** in 74% overall yield. Treatment of **7** with boron tribromide in dichloromethane gave the desired triphenolic diacid **8** in 52% yield and **6** in 41% yield which could be recycled. In this case, the demethylation occurred competitively with the more hindered benzyl groups, which, since they are more labile than the methoxy group, are then subsequently removed. Complete demethylation of the dimethyl ester of **6** under the above conditions was unsuccessful, only ester hydrolysis being observed. The triphenolic diacid **8** was converted into the pentabenzyl compound **9** in 42% yield selective hydrolysis of which gave **10** in 91% yield. This compound was identical spectroscopically with an authentic sample prepared by a different route.[7d,8] The tetrabenzyl compound **10** is suitably protected for activation and coupling to a protected portion of the azepane **21** to give balanol.

The synthesis of the azepane portion of balanol started from commercially available piperidin-4-one. *N*-Tosylation under standard conditions followed by bromination gave **11** in 80% overall yield. The tosyl group was chosen to protect the nitrogen as this group was more stable to the bromination conditions and subsequent stages than carbamates and all compounds containing the tosyl group were crystalline. It was expected that the tosyl group would have to be removed and replaced by a more labile group after the requisite functional group manipulations had been completed so that deprotection at the final stage of the balanol synthesis would not be problematic.

The regiospecific homologation of unhindered α-bromo ketones using ethyl diazoacetate and boron trifluoride–diethyl ether has been reported.[11] The observed regioselectivity of homologation has been demonstrated to be due to a combination of steric and electronic factors.[11] The electron-withdrawing effect of the α-bromo substituent suppresses the migration of the carbon atom bearing it and the steric effect of the bromine atom enhances this selectivity of migration. This



Scheme 1 *Reagents and conditions:* i, Mg, THF, **2** (80%); ii, $KMnO_4$, pyridine (aq.) (38%); iii, $SOCl_2$, MeOH, iv, BBr_3, CH_2Cl_2 (95% from **5**); v, $SOCl_2$, MeOH; vi, NaH, BnBr, DMF (74% from **6**); vii, BBr_3, CH_2Cl_2 (52%); viii, NaH, BnBr, DMF (42%); ix, Na_2CO_3 (aq.), EtOH (91%)



Scheme 2 *Reagents and conditions:* i, N_2CHCO_2Et, BF_3-Et_2O, CH_2Cl_2 (71%); ii, HCl (aq), dioxane (90%); iii, NaN_3, AcOH, DMF (73%); iv, $NaBH_4$, EtOH (88%); v, PPh_3, DIAD, THF, *p*-nitrobenzoic acid (84%); vi, NaOH (aq.), MeOH, dioxane (99%); vii, $LiAlH_4$, THF (85%); viii, HBr (aq.) (68%); ix, Et_3N, CH_2Cl_2, 18-crown-6, benzyl chloroformate (88%); x, 4-(benzyloxy)benzoyl chloride, Et_3N, CH_2Cl_2 (65%)

sort of regiospecific homologation has not been applied previously to unsymmetrical ketones present in heterocyclic systems. A novel use of this type of ring expansion was demonstrated in the conversion of **11** into **12** using ethyl diazoacetate under Lewis acid conditions, which proceeded in 71% yield. As predicted, insertion of the methylene was selective for the unhindered side of the ketone, with none of the other regioisomer being observed. Hydrolysis and decarboxylation of **12** gave **13** in 90% yield. Reaction of **13** with sodium azide resulted in nucleophilic displacement of bromide by azide to give **14** in 73% yield. Reduction of **14** with sodium boranuide ($NaBH_4$) gave a *cis*:*trans* (2.4:1) mixture of azido alcohols **16** and **15** which were easily separable by chromatography giving **15** in 26% and **16** in 62% yields, respectively. Conversion of the major unwanted *cis* isomer **16** into the *trans* isomer **15** proceeded in 84% yield using a Mitsunobu[12] inversion *via* the *p*-nitrobenzoate **17** followed by hydrolysis. The *trans* relationship of the hydroxy and azido groups in **15** was unambiguously established by X-ray crystallography at this stage as shown in Fig. 1. Treatment of **15** with lithium aluminium hydride gave the 3-amino-4-hydroxyazepane **18** in 85% yield. At this stage, the *N*-tosyl group had to be replaced by a group that would be more easily removed in the final stage of the synthesis. Removal of the *N*-tosyl group was effected in 68% yield using aqueous hydrobromic acid to give **19**. Selective protection of the secondary amino group in **19** proceeded in 88% yield using benzyl chloroformate in the presence of 18-crown-6 to give **20**. Reaction of **20** under standard conditions with 4-(benzyloxy)benzoyl chloride gave **21** in 65% yield, which is a suitably protected azepane portion of balanol to be coupled to **10** following activation. The enantiomers of **21** were resolved by forming their Mosher's esters[13] **22** followed by chromatography and hydrolysis to give both enantiomers of **21** in

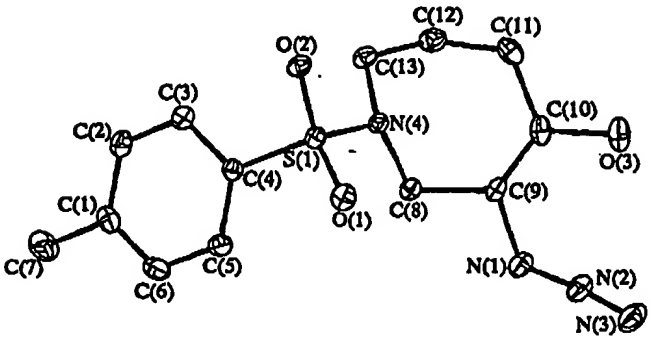
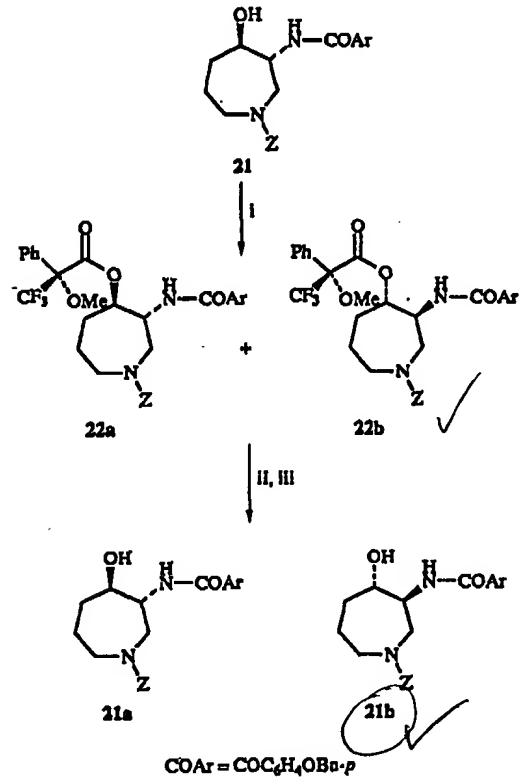


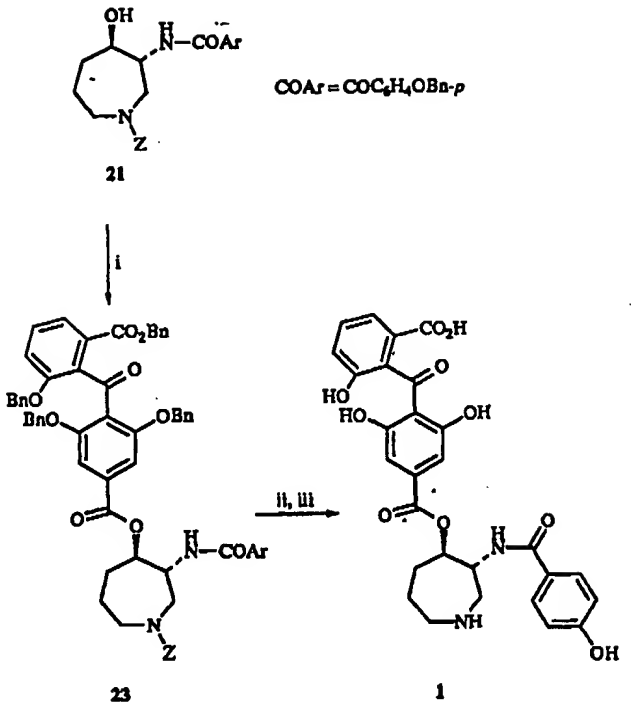
Fig. 1 X-Ray structure of compound 15



Scheme 3 *Reagents and conditions:* i, acid chloride of (*S*)-(-)-MTPA; CH_2Cl_2, Et_3N, DMAP; ii, chromatography (96% from **21**); iii, KOH, MeOH (100%)

96% overall yield and in >99% ee. The enantiomeric purity was checked relative to the racemate using chiral chromatography. This separation allowed access to both enantiomerically pure forms of balanol in a similar manner to that previously described.[7a] Compound **21** was identical with an authentic sample prepared by a different route.[8]

Activation of **10** by the method of Mukaiyama,[14] as employed in the Nicolaou synthesis[8] of balanol followed by coupling to **21** gave **23** which was identical spectroscopically with an authentic sample.[8] Hydrogenolysis of **23** with palladium black in aqueous acetic acid–ethyl acetate gave balanol **1** as a major product. In our hands the use of THF as a co-solvent under similar conditions to Nicolaou during the deprotection step gave rise to quantities of *N*-hydroxybutylbalanol. This side reaction was avoided by replacing the THF with ethyl acetate. Purification of **1** using reverse phase HPLC gave pure balanol **1** identical by HPLC, MS and NMR with an authentic sample of balanol.[8]



Scheme 4 *Reagents and conditions:* i, **10**, 2-chloro-1-methylpyridinium iodide, DMAP, Et_3N, CH_2Cl_2 (37%); ii. H_2, Pd black, HOAc, EtOAc, H_2O; iii, HPLC (53% from **23**)

Experimental

Mps were determined using an Electrothermal apparatus and are uncorrected. IR spectra were recorded on a Nicolet 20SXB spectrophotometer. ^1H NMR spectra were obtained using a Varian VXR400 instrument (400 MHz), δ values quoted are relative to internal TMS and J values are given in Hz. Mass spectra were measured with either a Varian VG 7070E or Finnegan TSQ 700 spectrometer. Flash chromatography was performed using Sorbsil C 60 (40–60 μm mesh) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (E. Merck Kieselgel 60 F_{254}) and compounds were visualised using UV fluorescence, ethanolic phosphomolybdic acid or aqueous potassium permanganate.

2,6-Dimethoxy-4-methylphenyl 2-methoxy-6-methylphenyl ketone 4

A suspension of magnesium turnings (0.6 g, 25 mmol) in THF (2 cm^3) under an atmosphere of nitrogen was treated with 1,2-dibromoethane (0.14 cm^3). The mixture was slowly stirred and heated to reflux whilst a solution of 2-bromo-3-methoxytoluene **3** (5.03 g, 25 mmol) in THF (20 cm^3) was added dropwise. The mixture was heated at reflux for 1 h, allowed to cool to room temperature and then filtered to remove any traces of magnesium. To this Grignard reagent a solution of 2,6-dimethoxy-4-methylbenzoyl chloride **3** (5.25 g, 25 mmol) in THF (20 cm^3) was added dropwise, the temperature of the mixture being kept at *ca.* 25 °C. The mixture was stirred at room temperature for 30 min after which the solvent was removed under reduced pressure to give a pale red syrup which was treated with saturated aq. NH_4Cl (200 cm^3) and ethyl acetate (150 cm^3). The layers were separated and the aqueous layer was extracted with ethyl acetate (50 cm^3). The combined organic layer and extracts were washed with 5% aq. $NaHCO_3$ (3 × 100 cm^3) and brine (100 cm^3) dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield a yellow solid (7.2

g). This was recrystallised from ethanol to yield 4 (6.0 g, 80%) as a white solid, mp 132–133 °C (Found: C, 71.8; H, 6.6. $C_{18}H_{20}O_4$ requires C, 72.0; H, 6.7%); ν_{max}(KBr)/cm^{-1} 1670s (CO); δ_H($CDCl_3$) 2.31 (3 H, s, Me), 2.34 (3 H, s, Me), 3.58 (3 H, s, 6-OMe), 3.66 (6 H, s, 2′- and 6′-OMe), 6.34 (2 H, s, 3′- and 5′-H), 6.67 (1 H, d, *J* 8), 6.78 (1 H, d, *J* 8) and 7.16 (1 H, t, *J* 8, 4-H); *m/z* 301 (MH^+).

4-Carboxy-2,6-dimethoxyphenyl 2-carboxy-6-methoxyphenyl ketone 5

To a stirred solution of potassium permanganate (28 g, 3 equiv.) in water (160 cm^3) and pyridine (160 cm^3) at *ca.* 100 °C, aq. NaOH (20%; 4 cm^3) was added. To this stirred mixture a solution of 4 (17.67 g, 16.98 mmol) in pyridine (90 cm^3) was added dropwise followed by water (90 cm^3). The mixture was heated at reflux for 1 h after which further quantities of potassium permanganate (28 g, 3 equiv.) were added hourly to it, stirring and heating being continued, until a total of 208 g, 24 equiv. had been added. The hot reaction mixture was filtered through Celite and the filter cake was washed with water (4 × 150 cm^3) and pyridine (50 cm^3). The filtrate was cooled on ice and acidified to pH 1 by the addition of concentrated aq. HCl; any precipitated solid was filtered off. The acidic aqueous filtrate was treated with NaCl (200 g) and extracted with ethyl acetate (6 × 400 cm^3). The combine/extracts were dried ($MgSO_4$), filtered and evaporated under reduced pressure and the residue treated with diethyl ether to give 5 (8.1 g, 38%) as a white solid, mp 253–256 °C (Found: C, 59.1; H, 4.5. $C_{18}H_{16}O_8$·0.25H_2O requires C, 59.25; H, 4.4%); ν_{max}(KBr)/cm^{-1} 3400br and 2900br (OH), 1690s (CO); δ_H(CD_3SOCD_3) 3.59 (3 H, s, 6-OMe), 3.64 (6 H, s, 2′- and 6′-OMe), 7.17 (2 H, s, 3′- and 5′-H), 7.22 (1 H, d, *J* 8), 7.29 (1 H, d, *J* 8) and 7.46 (1 H, t, *J* 8, 4-H); *m/z* 361 (MH^+).

4-Carboxy-2-hydroxy-6-methoxyphenyl 2-carboxy-6-hydroxyphenyl ketone 6

To a stirred solution of 5 (1.01 g, 2.8 mmol) in methanol (25 cm^3), thionyl chloride (1.17 cm^3, 16 mmol) was added dropwise and the mixture heated at reflux for 2 h. The mixture was allowed to cool to room temperature and then evaporated to dryness. Treatment of the residue with toluene (2 × 20 cm^3) and removal of the solvent gave a white solid (1.09 g) which was dissolved in dichloromethane (37 cm^3) and the resulting solution was cooled to −60 °C. To the solution at −60 °C boron tribromide in dichloromethane (1 mol dm^{-3}; 31 cm^3) was added dropwise and the mixture was allowed to warm to room temperature with stirring for 18 h. The mixture was cooled to 0 °C and water (50 cm^3) was added dropwise to it. The mixture was allowed to warm to room temperature and any precipitated solid was removed by filtration. The layers were separated and the aqueous layer was adjusted to pH 4 by the addition of aq. $NaHCO_3$ (5%) and the aqueous layer was re-extracted with ethyl acetate (2 × 50 cm^3). The solid removed by the previous filtration was dissolved in the combined organic extracts and the solution dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield 6 (0.9 g, 95%) as a pale yellow solid, mp 262–265 °C (Found C, 57.8; H, 3.7. $C_{16}H_{12}O_8$ requires C, 57.8; H, 3.6%); ν_{max}(KBr)/cm^{-1} 3490br, 3415br and 2900br (OH), 1680s and 1635s (CO); δ_H(CD_3SOCD_3) 3.49 (3 H, s, 6′-OMe), 6.90 (1 H, s), 7.05 (1 H, s), 7.08 (1 H, d, *J* 8), 7.32 (1 H, t, *J* 8, 4-H), 7.39 (1 H, d, *J* 8), 9.90 (1 H, br s, OH) and 12.75 (1 H, br s; OH); *m/z* 333 (MH^+).

2-Hydroxy-6-methoxycarbonylphenyl 2-hydroxy-6-methoxy-4-methoxycarbonylphenyl ketone

To a stirred solution of 6 (5.64 g, 17 mmol) in methanol (200 cm^3) thionyl chloride (12 cm^3, 165 mmol) was added dropwise. The mixture was heated at reflux for 3 h, after which it was allowed to cool to room temperature and then evaporated to dryness to give a light brown crystalline solid. This was then treated first with toluene (2 × 20 cm^3) which was evaporated under reduced pressure and then with diethyl ether to give a cream crystalline solid (6.13 g, 100%), mp 154–156 °C (Found C, 59.8; H, 4.5. $C_{16}H_{16}O_8$ requires C, 60.0; H, 4.5%); ν_{max}(KBr)/cm^{-1} 3450br (OH), 1725s and 1710s (CO); δ_H($CDCl_3$) 3.46 (3 H, s, 6′-OMe), 3.52 (3 H, s, CO_2Me), 3.83 (3 H, s, CO_2Me), 6.94 (1 H, s), 7.12 (1 H, d, *J* 8), 7.30 (1 H, s), 7.38 (1 H, t, *J* 8, 4-H), 7.44 (1 H, d, *J* 8); *m/z* 361 (MH^+).

2-Benzyloxy-6-methoxycarbonylphenyl 2-benzyloxy-6-methoxy-4-methoxycarbonylphenyl ketone 7

To a stirred solution of 2-hydroxy-6-methoxycarbonylphenyl 2-hydroxy-6-methoxy-4-methoxycarbonylphenyl ketone (6.13 g, 17 mmol) in dry DMF (120 cm^3) at 0 °C NaH (60% in oil; 2.04 g, 51 mmol) was added during 3 min. After the mixture had been allowed to warm to room temperature with stirring over 1 h benzyl bromide (6.5 cm^3, 51 mmol) was added to it and the reaction mixture was heated at 65 °C for 2 h. After the mixture had been cooled to 0 °C it was treated with methanol (10 cm^3) and then evaporated under reduced pressure; the residue was then treated with ice water (400 cm^3) and extracted with diethyl ether (2 × 150 cm^3). The combined extracts were dried ($MgSO_4$) filtered and evaporated under reduced pressure to yield a yellow solid. Treatment of this with diethyl ether gave 7 (7 g, 74%) as a cream solid, mp 155–158 °C (Found C, 71.1; H, 5.1. $C_{32}H_{28}O_8$ requires C, 71.1; H, 5.2%); ν_{max}(KBr)/cm^{-1} 1730s and 1670s (CO); δ_H($CDCl_3$) 3.64 (3 H, s, 6′-OMe), 3.70 (3 H, s, CO_2Me), 3.95 (3 H, s, CO_2Me), 4.75 (2 H, s, OCH_2Ph), 4.86 (2 H, s, OCH_2Ph), 6.94 (3 H, m), 7.05 (2 H, m), 7.10 (1 H, s) and 7.15–7.35 (9 H, m); *m/z* 541 (MH^+).

4-Carboxy-2,6-dihydroxyphenyl 2-carboxy-6-hydroxyphenyl ketone 8

To a stirred solution of 7 (6 g, 11.1 mmol) in dichloromethane (150 cm^3) at −60 °C boron tribromide in dichloromethane (1 mol dm^{-3}; 122 cm^3) was added dropwise during 45 min. The reaction mixture was allowed to warm to room temperature after which it was kept at ambient temperature for 2 days. It was then cooled to 10 °C, treated dropwise with water (150 cm^3) and stirred at room temperature for 1 h. The layers were separated and the aqueous layer adjusted to pH 4 by the addition of solid $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (2 × 100 cm^3) and the combined extracts were dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield a yellow solid. The residue was purified by column chromatography on silica, eluting with methanol–dichloromethane (5:95, v/v), to give 8 (1.85 g, 52% uncorrected) as a light yellow solid, mp 222–225 °C (Found C, 56.3; H, 3.2. $C_{15}H_{10}O_8$ requires C, 56.6; H, 3.2%); ν_{max}(KBr)/cm^{-1} 3500br, 3100br, 2900br and 2600br (OH), 1700s and 1640s (CO); δ_H(CD_3SOCD_3) 6.69 (2 H, s, 2′- and 6′-H), 7.06 (1 H, d, *J* 8), 7.27 (1 H, t, *J* 8, 4-H) and 7.48 (1 H, d, *J* 8; *m/z* 318 (M^+). A quantity of 6 was also recovered (1.5 g, 41%) which could be recycled.

2-Benzyloxy-6-benzyloxycarbonylphenyl 2,6-dibenzyloxy-4-benzyloxycarbonylphenyl ketone 9

To a stirred solution of 8 (0.4 g, 1.26 mmol) in dry DMF (30 cm^3) at 0 °C NaH (60% in oil; 0.3 g, 7.56 mmol) was added portionwise and the mixture was allowed to warm to room temperature with stirring for 1 h. After treatment with benzyl bromide (0.9 cm^3, 7.57 mmol), the mixture was heated at 65 °C for 20 h. The mixture was cooled to 0 °C and treated with a further quantity of NaH (0.15 g, 3.78 mmol) after which it was stirred at room temperature for 30 min, and then treated with benzyl bromide (0.9 cm^3, 7.57 mmol). After being heated at 65 °C for a further 4 h, the mixture was cooled to 0 °C and

treated with methanol (10 cm^3); it was then evaporated under reduced pressure. The residue was treated with water (60 cm^3) and then extracted with ethyl acetate (3 × 50 cm^3). The combined extracts were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was chromatographed on a Dynamax silica column (20 mm × 300 mm) eluting with heptane–ethyl acetate (85:15, v/v) to yield **9** (0.4 g, 42%) as a white solid, mp 127–130 °C (Found C, 77.9; H, 5.1. $C_{50}H_{40}O_8$ requires C, 78.1; H, 5.2%); ν_{max}(KBr)/cm^{-1} 1720s and 1680s (CO); δ_H($CDCl_3$) 4.70 (2 H, s, 6-OCH_2Ph), 4.78 (4 H, s, 2'- and 6'-OCH_2Ph), 5.12 (2 H, s, 2-$CO_2C H_2$Ph), 5.38 (2 H, s, 4'-$CO_2C H_2$Ph), 6.82 (2 H, m), 6.95 (1 H, d, *J* 8) and 7.01–7.48 (27 H, m); *m/z* 769 (MH^+).

2-Benzyloxy-6-benzyloxycarbonylphenyl 2,6-dibenzyloxy-4-carboxyphenyl ketone 10

To a stirred mixture of **9** (0.4 g, 0.52 mmol) in ethanol (30 cm^3), a solution of Na_2CO_3 (0.24 g, 1.04 mmol) in water (30 cm^3), was added and the mixture heated at reflux for 4 h. The mixture was allowed to cool to room temperature after which the ethanol was removed under reduced pressure. Water (150 cm^3) was added to the residue and the solution adjusted to pH 1 by the addition of concentrated aq. HCl. The aqueous mixture was then extracted with ethyl acetate (3 × 50 cm^3) and the combined extracts were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to yield a pale yellow solid. Trituration of this with diethyl ether gave **10** (320 mg, 91%) as a white solid, mp 132–134 °C; δ_H($CDCl_3$) 4.72 (2 H, s, 6-OCH_2Ph), 4.80 (4 H, s, 2'- and 6'-OCH_2Ph), 5.14 (2 H, s, 2'-$CO_2C H_2$Ph), 6.85 (2 H, d, *J* 8), 6.97 (1 H, d, *J* 8), 7.05–7.08 (4 H, m), 7.13 (2 H, t, *J* 8, 4-H) and 7.18–7.34 (16 H, m); *m/z* 679 (MH^+). Spectroscopic data of **10** were identical with those reported.[7d,8]

3-Bromo-1-(4'-methylphenylsulfonyl)piperidin-4-one 11

To a solution of 1-(4'-methylphenylsulfonyl)piperidin-4-one (262.2 g, 1.036 mol) in dichloromethane (6.5 dm^3) at −5 °C, a solution of bromine (51.8 cm^3, 1.004 mol) in dichloromethane (1 dm^3) was added dropwise during 2 h. The temperature of the mixture was maintained between −4 and −2 °C during the first 90 min of the addition and allowed to rise to 0 °C during the last 30 min. The resulting solution was allowed to warm to room temperature with stirring over 1 h. Saturated aq. $NaHCO_3$ (2 dm^3) was added to the reaction mixture followed by water (2 dm^3) and the resulting biphasic system was stirred for 30 min; the layers were then separated. The organic phase was extracted with aq. $NaHCO_3$ (2.5 dm^3) and then dried, ($MgSO_4$), filtered and evaporated under reduced pressure to give **11** (340 g, 98%) as a white solid, mp 129–134 °C (Found: C, 43.2; H, 4.2; N, 4.3. $C_{12}H_{14}BrNO_3S$ requires C, 43.4; H, 4.25; N, 4.2%); ν_{max}(KBr)/cm^{-1} 1735s (CO), 1340s and 1160s (SO_2); δ_H($CDCl_3$) 2.45 (3 H, s, 4'-Me), 2.66 (1 H, dddd, *J* 14.8, 8.9, 5.7 and 1.2, 5-H_{ax}), 2.96 (1 H, ddd, *J* 14.8, 5.7 and 4.5, 5-H_{eq}), 3.25 (1 H, dddd, *J* 12.4, 8.9, 4.5 and 1.2, 6-H_{ax}), 3.36 (1 H, ddd, *J* 12.8, 8.4 and 1.2, 2-H_{ax}), 3.65 (1 H, dtd, *J* 12.4, 5.7 and 1.8, 6-H_{eq}), 3.98 (1 H, ddd, *J* 12.8, 5.1 and 1.8, 2-H_{eq}), 4.55 (1 H, ddd, *J* 8.4, 5.1 and 1.2, 3-H), 7.36 (2 H, d, *J* 8, 3'- and 5'-H) and 7.7 (2 H, d, *J* 8, 2'- and 6'-H); *m/z* 331 and 333 (M^+).

3-Bromo-5-ethoxycarbonyl-1-(4'-methylphenylsulfonyl)azepan-4-one 12

To a solution of **11** (25.1 g, 75.6 mmol) in dichloromethane (822 cm^3) at −5 °C under nitrogen, a solution of boron trifluoride–diethyl ether (9.96 cm^3, 79.3 mmol) in dichloromethane (117 cm^3) was added dropwise over 15 min, the temperature of the mixture being kept between −5 and −3 °C. The solution was stirred at −5 °C for 20 min after which a solution of ethyl diazoacetate (9.93 cm^3, 94.4 mmol) in dichloromethane (117 cm^3) was added dropwise to it during 20 min, the temperature of the solution being maintained between −5 and −2 °C during the addition. The solution was allowed to warm to room temperature for 90 min, after which it was diluted with water (234 cm^3) and then stirred at room temperature for 25 min. The layers were separated and the organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a pale yellow oily solid which was crystallised from ethyl acetate to yield **12** (22.4 g, 71%) as a colourless solid, mp 165–167 °C (Found: C, 45.6; H, 4.75; N, 3.2. $C_{16}H_{20}BrNO_5S$ requires C, 45.9; H, 4.8; N, 3.3%); ν_{max}(KBr)/cm^{-1} 1740s and 1710s (CO), 1340s and 1160s (SO_2); δ_H($CDCl_3$) 1.26 (3 H, t, CH_3CH_2O), 2.05 (1 H, dtd, *J* 14.7, 12.1 and 4.9, 6-H_b), 2.20 (1 H, dq, *J* 14.7 and 2.9, 6-H_a), 2.45 (3 H, s, 4'-Me), 2.81 (1 H, ddd, *J* 14.0, 12.1 and 2.9, 7-H_b), 3.07 (1 H, dd, *J* 15.3 and 11.0, 2-H_b), 4.00 (1 H, dddd, *J* 14.0, 4.9, 2.9 and 0.9, 7-H_a), 4.02 (1 H, dd, *J* 12.1 and 2.9, 5-H_a), 4.19 (2 H, q, CH_3CH_2O), 4.27 (1 H, m, *J* 15.3, 6.5 and 0.9, 2-H_a), 4.45 (1 H, dd, *J* 11.0 and 6.5, 3-H), 7.34 (2 H, d, *J* 8, 3'- and 5'-H) and 7.67 (2 H, d, *J* 8, 2'-and 6'-H); *m/z* 418 and 420 (MH^+).

3-Bromo-1-(4'-methylphenylsulfonyl)azepan-4-one 13

To a suspension of **12** (45.4 g, 0.109 mol) in 1,4-dioxane (680 cm^3) at 80 °C aq. HCl (3 mol dm^{-3}; 364 cm^3) was added during 10 min. The resulting solution was heated at reflux for 7 h, cooled to room temperature and kept for 16 h. After this it was evaporated under reduced pressure to give a pale brown solid which was dissolved in ethyl acetate (650 cm^3) and the solution washed with water (2 × 50 cm^3) and brine (50 cm^3) dried (Na_2SO_4), filtered, and evaporated under reduced pressure to afford a pale brown crystalline solid. This was recrystallised from diisopropyl ether to give **13** (33.9 g, 90%) as a colourless crystalline solid, mp 104–106 °C (Found: C, 45.2; H, 4.7; N, 4.0 $C_{13}H_{16}BrNO_3S$ requires C, 45.1; H, 4.7; N, 4.05%); ν_{max}(KBr)/cm^{-1} 1710s (CO), 1340s and 1160s (SO_2); δ_H($CDCl_3$) 1.87 (1 H, m, 6-H_b), 1.96 (1 H, m, 6-H_a), 2.45 (3 H, s, 4'-Me), 2.56 (1 H, ddd, *J* 12.4, 6.8 and 2.6, 5-H_b), 2.77 (1 H, ddd, *J* 13.7, 11.7 and 3.4, 7-H_b), 2.88 (1 H, td, *J* 12.4 and 3.4, 5-H_a), 3.05 (1 H, dd, *J* 15.1 and 11.0, 2-H_b), 4.0 (1 H, dt, *J* 13.7 and 3.8, 7-H_a), 4.24 (1 H, ddd, *J* 15.1, 6.4 and 1.10, 2-H_a), 4.38 (1 H, dd, *J* 11.0 and 6.4, 3-H), 7.34 (2 H, d, *J* 8, 3'- and 5'-H) and 7.67 (2 H, d, *J* 8, 2'- and 6'-H); *m/z* 346 and 348 (MH^+).

3-Azido-1-(4'-methylphenylsulfonyl)azepan-4-one 14

To a solution of **13** (33.9 g, 97.9 mmol) in dry DMF (750 cm^3) under argon, acetic acid (11.2 cm^3) and then sodium azide (12.7 g, 0.195 mol) were added. The resulting suspension was stirred at room temperature for 3.5 h and then kept at room temperature for 16 h. Upon dilution of the mixture with water (1.875 dm^3) an oily solid separated and this was extracted with ethyl acetate (1 × 900 cm^3, 3 × 300 cm^3). The combined extracts were washed with brine (225 cm^3) dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield a yellow viscous liquid which crystallised when kept at room temperature. Recrystallisation of this from *tert*-butyl methyl ether gave **14** (21.9 g, 73%) as a white crystalline solid, mp 88 °C (Found: C, 50.3; H, 5.15; N, 17.8. $C_{13}H_{16}N_4O_3S$ requires C, 50.6; H, 5.2; N, 18.1%); ν_{max}(KBr)/cm^{-1} 2090s (N_3), 1710s (CO), 1340s and 1160s (SO_2); δ_H($CDCl_3$) 1.89 (1 H, dddd, *J* 14.8, 8.2, 6.5 and 4.2, 6-H_b), 1.95 (1 H, m, 6-H_a), 2.45 (3 H, s, 4'-Me), 2.64 (1 H, ddd, *J* 13.8, 9 and 4.2, 5-H_b), 2.69 (1 H, ddd, *J* 13.8, 8.2 and 4.2, 5-H_a) 3.02 (1 H, ddd, *J* 13.7, 8.2 and 4.2, 7-H_b), 3.04 (1 H, dd, *J* 14.8 and 8.7, 2-H_b), 3.68 (1 H, dddd, *J* 13.7, 6.5, 4.2 and 1, 7-H_a), 3.78 (1 H, ddd, *J* 14.8, 5.3 and 1, 2-H_a), 4.21 (1 H, dd, *J* 8.7 and 5.3), 7.34 (2 H, d, *J* 8, 3'- and 5'-H) and 7.68 (2 H, d, *J* 8, 2'- and 6'-H).

***trans*-3-Azido-1-(4'-methylphenylsulfonyl)azepan-4-ol 15 and *cis*-3-azido-1-(4'-methylphenylsulfonyl)azepan-4-ol 16**

To a suspension of **14** (21.8 g, 70.7 mmol) in ethanol (220 cm^3)

at 0 °C sodium boranuide (2.67 g, 70.06 mmol) was added portionwise over 10 min. The resulting clear solution was stirred at 2–5 °C for 15 min after which it was cooled in an ice–water bath whilst water (670 cm^3) was added to it followed by aq. HCl (1 mol dm^{-3}; 110 cm^3), added dropwise over 10 min as the mixture was allowed to warm to *ca.* 20 °C. The mixture was then stirred at *ca.* 20 °C for 15 min after which it was further diluted with water (440 cm^3) to give separation of a yellow oil which was extracted with ethyl acetate (1 × 440 cm^3, 3 × 220 cm^3). The combined extracts were washed with brine (2 × 55 cm^3), dried (Na_2SO_4), filtered and evaporated under reduced pressure. The products were purified on SiO_2 eluting with *tert*-butyl methyl ether–pentane (1:1, v/v to 7:3, v/v): to give **16** (13.5 g, 62%) as a white solid, mp 87–90 °C (Found: C, 50.15; H, 5.75; N, 18.15. $C_{13}H_{18}N_4O_3S$ requires C, 50.31; H, 5.85; N, 18.05%); ν_{max}(KBr)/cm^{-1} 3510br (OH), 2940s (N_3), 1340s and 1160s (SO_2); δ_H($CDCl_3$) 1.73 (2 H, m, 6-H), 1.98 (2 H, m, 5-H), 2.05 (1 H, dd, *J* 3.6 and 1.2, -OH), 2.45 (3 H, s, 4′-Me), 2.93 (1 H, dt, *J* 12.4 and 5.9, 7-H_b), 3.08 (1 H, dd, *J* 14.4 and 9.6, 2-H_b), 3.61 (2 H, m, 2-H_a and 7-H_a), 3.78 (1 H, ddd, *J* 9.6, 3.9 and 3.3, 3-H), 4.1 (1 H, br m, 4-H), 7.34 (2 H, d, *J* 8, 3′- and 5′-H) and 7.68 (2 H, d, *J* 8, 2′- and 6′-H); and **15** (5.7 g, 26%) as a white solid, mp 67–70 °C (Found: C, 50.2; H, 5.8; N, 18.1. $C_{13}H_{18}N_4O_3S$ requires C, 50.3; H, 5.85; N, 18.05%); ν_{max}(KBr)/cm^{-1} 3510br (OH), 2940s (N_3), 1340s and 1160s (SO_2); δ_H($CDCl_3$) 1.54 (1 H, m, 6-H_b), 1.78 (1 H, m, 6-H_a), 1.96 (2 H, m, 5-H), 2.22 (1 H, d, *J* 3.9, OH), 2.45 (3 H, s, 4′-Me), 2.82 (1 H, dd, *J* 14.8 and 9, 2-H_b), 3.01 (1 H, ddd, *J* 12.2, 6.5 and 3.5, 7-H_b), 3.48 (2 H, m, 4-H and 7-H_a), 3.53 (1 H, dd, *J* 9 and 3.6, 3-H), 3.66 (1 H, ddd, *J* 14.8, 3.6 and 0.9, 2-H_a), 7.34 (2 H, d, *J* 8, 3′- and 5′-H) and 7.68 (2 H, d, *J* 8, 2′- and 6′-H).

trans-3-Azido-1-(4′-methylphenylsulfonyl)-4-(4″-nitrobenzoyloxy)azepane 17

To a solution of triphenylphosphine (17.6 g, 67.1 mmol) in dry THF (330 cm^3) at 5 °C, DIAD (diisopropylazodicarboxylate; 13.9 cm^3, 67.1 mmol) was added dropwise during 10 min while the temperature of the mixture was kept at 5–10 °C. A thick cream suspension formed which was stirred for a further 15 min and to this 4-nitrobenzoic acid (11.2 g, 67 mmol) and **16** (13.4 g, 43.2 mmol) were added. The resulting yellow solution was allowed to warm to room temperature as it was stirred for 1 h and then kept at room temperature for 16 h. After this the mixture was evaporated under reduced pressure and the residue stirred with *tert*-butyl methyl ether (170 cm^3) to yield a cream solid. The product was crystallised from *tert*-butyl methyl ether to give **17** (16.9 g, 85%) as colourless crystals, mp 164–168 °C (Found: C, 52.3; H, 4.6; N, 15.1. $C_{20}H_{21}N_5O_6S$ requires C, 52.3; H, 4.6; N, 15.2%); ν_{max}(KBr)/cm^{-1} 2105s (N_3), 1720s (CO), 1530s and 1350w (NO_2), 1330s and 1160s (SO_2); δ_H($CDCl_3$) 1.70 (1 H, m, 6-H_b), 2.06 (3 H, m, 5-H and 6-H_a), 2.45 (3 H, s, 4′-Me), 2.82 (1 H, dd, *J* 15 and 10, 2-H_b), 2.97 (1 H, ddd, *J* 12.3, 6.6 and 3.5, 7-H_b), 3.64 (1 H, ddd, *J* 12.3, 9.8 and 6.6, 7-H_a), 3.76 (1 H, ddd, *J* 15, 4.2 and 1, 2-H_a), 4.02 (1 H, ddd, *J* 10, 8.4 and 4.2, 3-H), 5.0 (1 H, ddd, *J* 10, 8.4 and 2.9, 4-H), 7.34 (2 H, d, *J* 8, 3′- and 5′-H), 7.7 (2 H, d, *J* 8, 2′- and 6′-H), 8.25 (2 H, d, *J* 8, 3″- and 5″-H) and 8.33 (2 H, d, *J* 8, 2″- and 6″-H).

Conversion of *trans*-3-azido-1-(4′-methylphenylsulfonyl)-4-(4″-nitrobenzoyloxy)azepane 17 into *trans*-3-azido-1-(4′-methylphenylsulfonyl)azepan-4-ol 15

To a suspension of **17** (16.9 g, 36.8 mmol) in methanol (750 cm^3) and 1,4-dioxane (190 cm^3) was added aq. NaOH (2% w/v; 150 cm^3) at room temperature. The suspension was stirred at room temperature for 5 h; a clear solution was obtained after 3 h. The solution was kept at room temperature for 16 h, after which it was evaporated under reduced pressure to give an oily residue which was stirred with ethyl acetate (460 cm^3) and water (460 cm^3). The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 230 cm^3). The combined organic layer and extracts were washed with brine (2 × 50 cm^3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a viscous liquid, which crystallised with time to give **15** (11.3 g, 99%) mp 66–70 °C, identical with **15** as prepared above.

***trans*-3-Amino-1-(4′-methylphenylsulfonyl)azepan-4-ol 18**

To a solution of **15** (16.9 g, 54.5 mmol) in dry THF (270 cm^3) at 0 °C under nitrogen, a solution of lithium aluminium hydride in THF (1 mol dm^{-3}; 27.3 cm^3, 27.3 mmol) was added dropwise during 10 min, while the temperature of the mixture was kept at 0–2 °C. After the mixture had been allowed to warm to room temperature it was stirred for 45 min. Water (30 cm^3) was then added dropwise during 10 min to the mixture with cooling (ice–water bath) followed by aq. Na_2CO_3 (275 cm^3; 20% w/v). The mixture was diluted with water (2 dm^3) and extracted with ethyl acetate (1 × 1 dm^3, 3 × 450 cm^3) and the combined extracts were washed with brine (2 × 150 cm^3), dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a colourless oil which crystallised with time. The product was recrystallised from *tert*-butyl methyl ether to give **18** (13.1 g, 85%) as colourless crystals, mp 93–96 °C (Found: C, 54.8; H, 7.05; N, 9.6. $C_{13}H_{20}N_2O_3S$ requires C, 54.9; H, 7.1; N, 9.85%); ν_{max}(KBr)/cm^{-1} 3360br (OH), 3100br and 2930br (NH_2), 1330s and 1150s (SO_2); δ_H($CDCl_3$) 1.64 (2 H, m, 6-H), 1.94 (2 H, m, 5-H), 2.45 (3 H, s, 4′-Me), 2.76 (1 H, td, *J* 8.1, 8.1 and 3.5, 3-H), 2.86 (1 H, dd, *J* 14.2 and 8.1, 2-H_b), 3.18 (1 H, ddd, *J* 12.6, 6.1 and 4.4, 7-H_b), 3.22 (1 H, m, 7-H_a), 3.29 (1 H, ddd, *J* 9.8, 8.1 and 2.4, 4-H), 3.49 (1 H, ddd, *J* 14.2, 3.5 and 0.6, 2-H_a), 7.31 (2 H, d, *J* 8, 3′- and 5′-H) and 7.66 (2 H, d, *J* 8, 2′- and 6′-H); *m/z* 285 (MH^+).

***trans*-3-Aminoazepan-4-ol dihydrobromide 19**

A suspension of **18** (13.0 g, 45.7 mmol) in aq. HBr (48%; 130 cm^3) was stirred and heated to reflux at which temperature the resulting yellow solution was kept for 3 h. The solution was then cooled to room temperature and kept for 16 h after which it was diluted with ice–water (260 cm^3) and then extracted with *tert*-butyl methyl ether (1 × 160 cm^3, 2 × 40 cm^3). The aqueous layer was concentrated under reduced pressure to give an oily yellow solid which was crystallised from ethanol to yield **19** (9.09 g, 68%) as a white crystalline solid, mp 215 °C (decomp.) (Found: C, 24.5; H, 5.6; N, 9.4. $C_6H_{16}Br_2N_2O$ requires C, 24.7; H, 5.5; N, 9.4%); ν_{max}(KBr)/cm^{-1} 3420br, 3230br and 2900br; δ_H(CD_3SOCD_3) 1.6–1.65 (3 H, m), 1.79–1.81 (1 H, m), 1.95–2.05 (1 H, m), 3.05–3.2 (3 H, m), 3.65 (1 H, br), 5.75 (1 H, br), 8.18 (3 H, br) and 9.1 (1 H, br).

***trans*-3-Amino-1-benzyloxycarbonylazepan-4-ol 20**

To a stirred suspension of **19** (9.01 g, 30.9 mmol) in dry dichloromethane (230 cm^3) at room temperature under nitrogen, triethylamine (21.5 cm^3) was added followed by 18-crown-6 (16.3 g, 61.7 mmol). The mixture was stirred at room temperature for 20 min and the resulting clear solution cooled to 5 °C and benzyl chloroformate (4.97 cm^3, 33.9 mmol) was added dropwise during 10 min. The mixture was stirred at room temperature for 5 h, allowed to stand at room temperature for 16 h and then concentrated under reduced pressure to give a pale yellow oily solid which was stirred with ethyl acetate (330 cm^3) and aq. Na_2CO_3 (10% w/v; 220 cm^3). The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 110 cm^3). The combined organic extracts were washed with aq. Na_2CO_3 (10% w/v 110 cm^3) and saturated aq. KBr (2 × 55 cm^3), dried (Na_2SO_4), filtered and the mixture was concentrated under reduced pressure to give a pale yellow oily solid. Crystallisation from *tert*-butyl methyl ether gave **20**

(7.19 g, 88%) as a colourless crystalline solid, mp 78–80 °C (Found: C, 63.7; H, 7.8; N, 10.7. $C_{14}H_{20}N_2O_3$ requires C, 63.6; H, 7.6; N, 10.6%); ν_{max}(KBr)/cm^{-1} 3350br, 3270br and 2900br, 1700s (CO); δ_H($CDCl_3$) 1.48 (1 H, m, 6-H_b), 1.61 (1 H, m, 6-H_a) 1.96 (2 H, m, 5-H), 2.66 (½ H, dt, *J* 9.9 and 3.7, 3-H), 2.70 (½ H, dt, *J* 9.9 and 3.7, 3-H), 2.87 (½ H, dd, *J* 14.4 and 9, 2-H_b), 2.97 (½ H, dd, *J* 14.4 and 9, 2-H_b), 3.22 (1½ H, m, 7-H_b and 4-H), 3.33 (½ H, ddd, *J* 13.6, 5.9 and 4.3, 7-H_b), 3.53 (½ H, ddd, *J* 13.6, 9.9 and 5.9, 7-Ha), 3.66 (½ H, ddd, *J* 13.6, 9.9 and 5.9, 7-Ha), 3.71 (½ H, dd, *J* 14.5 and 3.8, 2-H_a), 3.79 (½ H, dd, *J* 14.5 and 3.8, 2-H_a), 5.12, 5.17 (AB system, 2 H, *J* 12, PhCH_2) and 7.28–7.4 (5 H, m, Ar); *m/z* 265 (MH^+).

***trans*-3-[4-(Benzyloxy)benzamido]-1-benzyloxycarbonylazepan-4-ol 21**

To a stirred suspension of **20** (3.09 g, 11 mmol) in dichloromethane (60 cm^3) at 0 °C, triethylamine (2.28 cm^3, 16.4 mmol) was added. While the temperature of the mixture was maintained at *ca.* 0 °C, 4-(benzyloxy)benzoyl chloride (3.02 g, 11 mmol) in dichloromethane (30 cm^3) was added dropwise after which the mixture was stirred at *ca.* 0 °C for a further 3 h. The mixture was then evaporated under reduced pressure and the residue dissolved in ethyl acetate (500 cm^3). The resulting solution was washed with water (2 × 60 cm^3) and brine (1 × 60 cm^3), dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield a viscous yellow oil. Chromatography of the oil on silica, eluting with *tert*-butyl methyl ether gave a colourless viscous oil, crystallisation of which from diisopropyl ether gave **21** (3.29 g, 63%) as a colourless solid, mp 132–134 °C (Found: C, 70.8; H, 6.4; N, 5.8. $C_{28}H_{30}N_2O_5$ requires C, 70.9; H, 6.4; N, 5.9%); ν_{max}(KBr)/cm^{-1} 3330br (OH), 2950br (NH) and 1700s (CO); δ_H($CDCl_3$) 1.66 (1 H, m, 6-H_b), 1.78–2.0 (3 H, m, 5- and 6-H_a), 2.78 (1 H, ddd, *J* 14, 13 and 4, 7-H_b), 3.35 (1 H, dd, *J* 15 and 5, 2-H_b), 3.78 (1 H, ddd, *J* 10, 6 and 2, 4-H), 4.07–4.23 (3 H, m, 2-H_a, 3-H and 7-H_a), 5.12 (2 H, s, OCH_2Ph), 5.14, 5.21 (AB system, 2 H, *J* 12, PhCH_2), 5.46 (1 H, br, s, OH), 7.02 (2 H, d, *J* 8, Ar), 7.28–7.47 (10 H, m, Ar), 7.82 (2 H, d, *J* 8, Ar) and 8.83 (1 H, br d, NH); *m/z* 475 (MH^+). Spectroscopic data of **21** were identical with those reported.[8]

Separation of the enantiomers of 21

To a solution of **21** (170 mg, 0.36 mmol), triethylamine (0.1 cm^3, 0.72 mmol) and DMAP (4-dimethylaminopyridine; 44 mg, 0.36 mmol) in dichloromethane (5 cm^3), the acid chloride derived from (*S*)-(-)-MTPA [α-methoxy-α-(trifluoromethyl)phenylacetic acid; 113 mg, 0.45 mmol] in dichloromethane (2 cm^3) was added dropwise at room temperature. The mixture was stirred at room temperature for 16 h after which it was evaporated under reduced pressure. The residue was chromatographed on silica eluting with ethyl acetate–pentane (1:3, v/v) to give the enantiomerically pure Mosher's esters **22a** (120 mg, 48%); *m/z* 691 (MH^+), and **22b** (120 mg, 48%); *m/z*; 691 (MH^+). Both **22a** and **22b** appear as a *ca.* 3:1 rotameric mixture by ^{1}H NMR. Separate solutions of both **22a** (120 mg, 0.17 mmol) and **22b** (120 mg, 0.17 mmol) in methanol (5 cm^3) were stirred and treated with aq. KOH (1 mol dm^{-3}; 2 cm^3). Each reaction mixture was stirred at room temperature for 16 h and then diluted with diethyl ether (30 cm^3) and water (20 cm^3). The organic layer was separated and evaporated under reduced pressure to yield a colourless gum, treatment of which with diisopropyl ether gave, as white solids, **21a** (80 mg, 100%), mp 113–114 °C, and **21b** (80 mg, 100%), mp 116–118 °C. Both **21a** and **21b** were identical spectroscopically with **21**. The enantiomeric purity of **21a** and **21b** was assessed relative to racemic **21** using chiral chromatography on a Chiral Pack AD column (4.6 mm × 250 mm) eluting with ethanol–heptane (15:85, v/v) which showed **21a** and **21b** both to be > 99% enantiomerically pure.

Protected balanol 23

To a stirred solution of **10** (180 mg, 0.265 mmol), triethylamine (0.74 cm^3, 5.3 mmol) and DMAP (16.4 mg, 0.133 mmol) in dichloromethane (9 cm^3), 2-chloro-1-methylpyridinium iodide (88 mg, 0.345 mmol) was added and the mixture stirred at room temperature for 1 h. Compound **21** (125 mg, 0.26 mmol) was added to the mixture which was then, stirred at room temperature for 20 h and finally heated at reflux for 2 h. After being cooled to room temperature the mixture was evaporated under reduced pressure and the residue dissolved in ethyl acetate (20 cm^3). The resulting solution was washed with aq. $NaHCO_3$ (5%; 2 × 50 cm^3), dried ($MgSO_4$), filtered and evaporated under reduced pressure. Purification of the residue on silica eluting with ethyl acetate–dichloromethane (5:95, v/v) gave **23** (0.11 g, 37%) as a glass; *m/z* 1135 (MH^+); δ_H($CDCl_3$) as a *ca.* 3:1 rotameric mixture 1.6–2.1 (4 H, m), 2.9 (1 H, m), 3.4 (1 H, m), 4.08–4.16 (2 H, m), 4.67 (2 H, s, OCH_2Ph), 4.7–4.88 (1 H, m), 4.82, 4.85 (2 H, AB system, *J* 12, OCH_2Ph), 5–5.15 (1 H, m), 5.04 (2 H, s, OCH_2Ph), 5.1 (4 H, s, OCH_2Ph), 5.26 (2 H, s, OCH_2Ph), 6.82 (2 H, d, *J* 8, Ar), 6.88–6.95 (4 H, m, Ar), 7.0–7.45 (32 H, m, Ar), 7.72 and 7.79 (*ca.* 3:1, 2 H, d, *J* 8, Ar). Spectroscopic data were identical with those reported;[8] the presence of rotamers precludes the exact assignment of all resonances.

Balanol 1

A solution of **23** (106 mg, 0.093 mmol) in ethyl acetate–acetic acid–water (19 cm^3, 16:2:1, v/v) was treated with palladium black (10.6 mg) under an atmosphere of hydrogen. The reaction mixture was initially warmed to 50 °C and then stirred at room temperature for 4 h. After this the mixture was filtered and fresh catalyst (10.6 mg) added, to the filtrate; the reaction mixture was then placed under a fresh atmosphere of hydrogen and stirred at room temperature for 24 h. The catalyst was filtered off and washed with a solvent mixture (16 cm^3) as used for the reaction. The mixture was concentrated and the residue was chromatographed on a Dynamax C_{18} column (20 × 300 mm) eluting with acetonitrile–water–trifluoroacetic acid (20:80:0.1, v/v) to give **1** (27 mg, 53%) as a light yellow powder; *m/z* 551 (MH^+); δ_H(CD_3OD) 1.84–2.12 (4 H, br m, 5- and 6-H), 3.42–2.98 (4 H, br m, 2- and 7-H), 4.32 (1 H, br m, 3-H), 5.29 (1 H, m, 4-H), 6.76 (2 H, d, *J* 8.7, 4′- and 6′-H), 6.80 (1 H, d, *J* 7.8, 11″-H), 6.92 (2 H, s, 3″- and 7″-H), 7.17 (1 H, t, *J* 7.8, 12″-H), 7.25 (1 H, d, *J* 7.8, 13″-H) and 7.60 (2 H, d, *J* 8.7, 3′-, 7′-H). Spectroscopic data were identical with those reported.[8]

X-Ray crystal structure determination of *trans*-3-azido-1-(4′-methylphenylsulfonyl)hexahydroazepan-4-ol 15

A single crystal of compound **15** (from *tert*-butyl methyl ether, appoximate size 0.18 × 0.28 × 0.16 mm), mounted in a Lindemann tube, was used for X-ray data collection.

Crystal data. $C_{13}H_{18}N_4O_3S$, M = 310.37, colourless prisms, orthorhombic, space group $Pn2_1a$, a = 8.7030(9), b = 11.3280(13), c = 15.097(2) Å (by least squares refinement of the setting angles for 250 reflections within θ = 2.25–27.16 °), V = 1488.4(3) Å^{-3}, Z = 4, D_c = 1.385 g cm^{-3}, T = 120 K, μ(Mo-Kα) = 2.33 cm^{-1}, F(000) = 656.

Data collection, structure solution and refinement. Data were collected on a FAST TV Area detector diffractometer following previously described methods.[15] From the ranges scanned, 6059 data were recorded (2.25 < θ < 27.16 °; index ranges $-10 \leqslant h \leqslant 10$, $-13 \leqslant k \leqslant 9$, $-18 \leqslant l \leqslant 10$) and merged to give 2618 unique [R(int) = 0.0532]. The structure was solved by direct methods[16] and refined on F_o^2 by full matrix least squares[17] using all unique data corrected for Lorentz and polarisation factors. All non-hydrogen atoms were anisotropic. The hydrogen atoms were inserted in idealised positions with U_{iso} set at 1.5 times U_{eq} of the parent. The weighting scheme

used was $w = 1/[\sigma^2(F_o)^2 + (0.0612P)^2]$, where $P = [\max(F_o)^2 + 2(F_c)^2]/3$; this gave satisfactory agreement analysis. Final R_1 (on F) and R_{w2} (on F_o^2) values were 0.0473 and 0.1067 for all 2616 data and 191 parameters. The corresponding R values were 0.0424 and 0.1008 for 2323 data with $I > 2\sigma(I)$. Sources of scattering factors are given in ref. 17.

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References

1 (*a*) Y. Nishizuka, *Nature (London)*, 1988, **334**, 661; (*b*) R. M. Bell and D. J. Burns, *J. Biol. Chem.*, 1991, **256**, 4661; (*c*) Y. Asaoka, S. Nakamura, K. Yoshida and Y. Nishizuka, *Trends Biochem. Sci.*, 1992, **17**, 414.
2 (*a*) M. Castanga, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa and Y. Nishizuka, *J. Biol. Chem.*, 1982, **257**, 7874; (*b*) A. Jackobovits, A. Rosenthal and D. J. Capan, *EMBO J.*, 1990, **9**, 1165.
3 D. Bradshaw, C. H. Hill, J. S. Nixon and S. E. Wilkinson, *Agents Actions*, 1993, **38**, 135.
4 P. Kulanthaivel, Y. F. Hallock, C. Boros, S. M. Hamilton, W. P. Janzen, L. M. Ballas, C. R. Loomis, J. B. Jiang, B. Katz, J. R. Steiner and J. Clardy, *J. Am. Chem. Soc.*, 1993, **115**, 6452.
5 Y. F. Hallock, W. P. Janzen, L. M. Ballas, P. Kulanthaivel and C. Boros, WOP 03730/1993.
6 S. Ohsima, M. Yanagisawa, A. Katoh, T. Fujii, T. Sano, S. Matsukuma, T. Fuyumai, M. Fujui, K. Watanabe, K. Yokose, M. Arisawa and T. Okuda, *J. Antibiotics*, 1994, **47**, 639.
7 (*a*) P. F. Hughes, J. W. Lampe, C. K. Biggers, S. H. Smith and J. T. Olson, presented at the 24th Medicinal Chemistry Symposium, University of Utah, 1994; (*b*) J. W. Lampe, P. F. Hughes, C. K. Biggers, S. H. Smith, J. T. Olson and H. Hong, presented at the 208th ACS National Meeting, Washington, 1994; (*c*) S. P. Hollinshead, J. B. Nichols, J. W. Wilson, C. K. Biggers and J. W. Lampe, presented at the 208th ACS National Meeting, Washington, 1994; (*d*) J. W. Lampe, P. F. Hughes, C. K. Biggers, S. H. Smith and H. Hong, *J. Org. Chem.*, 1994, **59**, 5147.
8 K. C. Nicolaou, M. E. Bunnage and K. Koide, *J. Am. Chem. Soc.*, 1994, **116**, 8402.
9 J. Kalamar, E. Steiner, E. Chardlais and T. Posternak, *Helv. Chim. Acta*, 1974, **57**, 2368.
10 D. H. Hall and J. M. Insole, *J. Chem. Soc., Perkin Trans. 2*, 1972, 1164.
11 V. Dave and E. W. Warnhoff, *J. Org. Chem.*, 1983, **48**, 2590.
12 O. Mitsunobu, *Synthesis*, 1981, 1.
13 J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543.
14 T. Mukaiyama, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 707.
15 J. A. Darr, S. R. Drake, M. B. Hursthouse and K. M. A. Malik, *Inorg. Chem.*, 1993, **32**, 5704.
16 G. M. Sheldrick, *Acta Crystallogr., Sect. A*, 1990, **46**, 467.
17 G. M. Sheldrick, SHELXL-93, University of Göttingen, Germany, 1993.

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Inhibitors of GABA Uptake. Syntheses and ^{1}H NMR Spectroscopic Investigations of Guvacine, (3*RS*,4*SR*)-4-Hydroxypiperidine-3-carboxylic Acid, and Related Compounds

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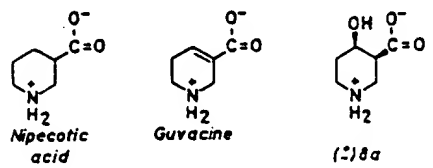
The syntheses of (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid (*8a*) and guvacine (1,2,5,6-tetrahydropyridine-3-carboxylic acid) hydrobromide (*9a*), both potent inhibitors of γ-aminobutyric acid (GABA) uptake, are described. Furthermore (3*RS*,4*SR*,5*SR*)- and (3*RS*,4*SR*,5*RS*)-4-hydroxy-5-methylpiperidine-3-carboxylic acids (*8c*) and (*8d*) and the guvacine analogues (*RS*)-5-methyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid (*10*) and 2,5,6,7-tetrahydro-1*H*-azepine-3-carboxylic acid and (1*R*,5*S*)-(−)-2-nortropene-2-carboxylic acid hydrobromides (*14*) and (*18*) have been synthesized. The compounds *8a,c,d*, *9a*, *10*, and *14* were prepared *via* catalytic hydrogenation of cyclic β-oxoesters and appropriate acid treatments of the intermediate β-hydroxy esters. Demethylation of ecgonine (*16*) followed by acid catalyzed hydrolysis and elimination reactions gave *18*. (*RS*)-Perhydroazepine-3-carboxylic acid and (1*R*,2*R*,5*R*)-(+)-nortropane-2-carboxylic acid hydrobromides (*15*) and (*19*) were obtained by catalytic hydrogenation of *14* and *18*, respectively. The relative stereochemistry of *6a,c,d*, *12*, *13*, and *19* was established by 270 MHz ^{1}H NMR spectroscopy. The relationship between structure and potency as inhibitors of GABA uptake of *8a,c,d*, *9a*, *10*, *14*, *15*, *18*, and *19* is discussed.



Scheme 1.

Nipecotic acid (piperidine-3-carboxylic acid),[1–3] guvacine (1,2,5,6-tetrahydropyridine-3-carboxylic acid),[4] and (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid (*8a*)[5] (Scheme 1) are potent substrate-competitive inhibitors of the neuronal γ-aminobutyric acid (GABA) uptake process. The concentrations of (*R*)-(−)-nipecotic acid, guvacine, and compound *8a* required for 50 % inhibition of GABA uptake (IC_{50} values) are 5 μM,[3] 8 μM,[4] and 12 μM,[5] respectively. These compounds seem to combine with the GABA transport carrier and penetrate the tissue.[2–4] Such compounds have pharmacological interest and may be useful tools for the study of the GABA transport carrier. However, molecular manipulations of these amino acids result in compounds with considerably reduced potency as inhibitors of GABA uptake, the IC_{50} values of *8c*, *8d*, *10*, and *15* being 260,[6] 349,[6] 547,[5] and 502 μM,[6] respectively. Compound *14*[6] and the conformationally rigid amino acids *18*[6] and *19*[5] are almost inactive. These findings demonstrate a pronounced substrate specificity of the GABA transport carrier.

This paper describes the syntheses of *8a*, its 5-methyl derivatives *8c* and *8d*, and the guvacine and nipecotic acid analoques *10*, *14*, *18*, *15*, and *19*. A convenient method for the preparation of guvacine hydrobromide (*9a*) has been developed. Guvacine hydrochloride has previously been synthesized on a very small scale.[7,8]

The β-oxoester *4b* was the only detectable product after Dieckmann cyclization of the unsymmetrical diester *3b* (Scheme 2). High pressure hydrogenation of *4a,b* gave the 3,4-*cis*-4-hydroxynipecotic acid derivatives *5a* and

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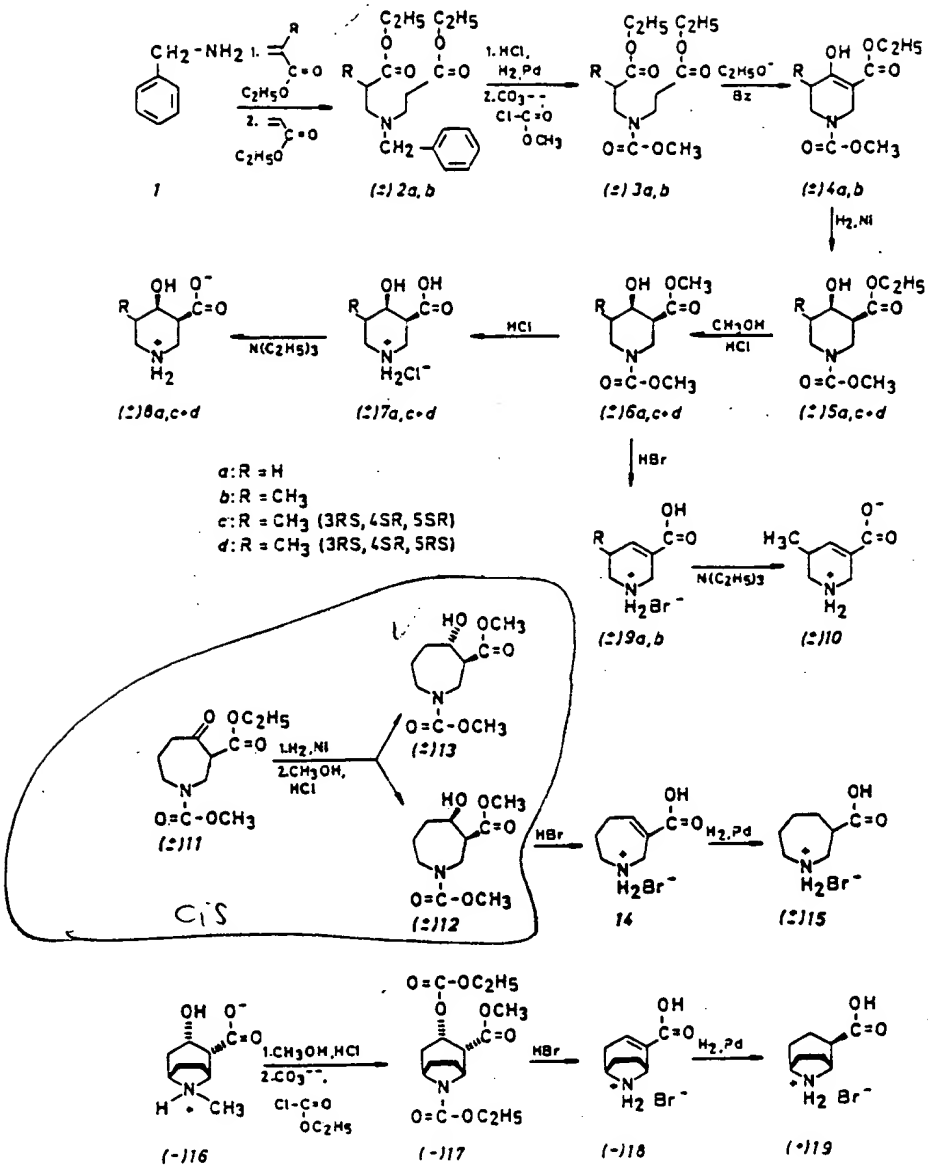
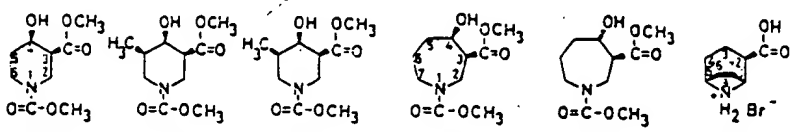


Scheme 2.

a separable mixture of *5c* and *5d*, respectively. The ethyl esters *5a,c,d* were transformed into the corresponding methyl esters in order to facilitate the analysis of the 270 MHz ^{1}H NMR spectra. Reduction of the *β*-oxoester *11*, however, gave a separable mixture of the *cis*- and *trans*-*β*-hydroxy esters *12* and *13* (Scheme 2). Appropriate treatmetnts of *6a,c,d* with hydrochloric acid gave the hydroxy amino acid chlorides *7a,c,d*, whereas the compounds *9a,b* were synthesized by prolonged treatments of *6a* and *6c* with hydrobromic acid. *N*-Demethylation of the methyl ester of ecgonine [(1*R*,2*R*, 3*S*,5*S*)-(−)-3-hydroxytropane-2-carboxylic acid]

Table 1. Some chemical shifts (δ) and coupling constants (Hz) from the 270 MHz ^{1}H NMR spectra of *6a,c,d*, *12*, *13*, and *19*.[a]



(±)6a (±)6c (±)6d (±)12 (±)13 (+)19

	(±)6a	(±)6c	(±)6d	(±)12	(±)13	(+)19
δ_{1e}	–	–	–	–	–	4.15
δ_{2a}	3.43	3.19	*ca.* 3.7	3.38	3.17, 3.22	2.88
δ_{2e}	4.05	4.28	*ca.* 3.7	3.92, 4.07	3.79, 3.96	
δ_{3a}	2.64	2.58	2.81	2.71, 2.78	2.60, 2.66	
δ_{4a}	–	–	–	–	3.91	
δ_{4e}	4.32	4.17	3.79	4.32	–	
δ_{5a}	1.66	1.68	–	1.53	1.62	
δ_{5e}	1.84	–	1.98	2.06	1.90	3.93
δ_{6a}	3.30	2.82	3.23	1.69	1.62	
δ_{6e}	3.79	3.80	3.59	2.06	2.00	
δ_{7a}	–	–	–	3.20, 3.25	3.32	
δ_{7e}	–	–	–	3.64	3.56	
δC–COOC*H*$_3$	3.68	3.69	3.69	3.71, 3.72	3.69	
δN–COOC*H*$_3$	3.71	3.73	3.72	3.74, 3.75	3.75	
δC–C*H*$_3$	–	0.99	0.98	–	–	
J_{1e2a}	–	–	–	–	–	2.2
J_{2a2e}	–13.2	–13.0		–14.5	–14.8	
J_{2a3a}	10.7	12.0	10.0[b]	10.2	9.0	
J_{2e3a}	4.5	2.0	2.6[b]	3.0	3.75	
J_{3a4e}	2.5	4.4	3.4	3.0	–	
J_{3a4a}	–	–	–	–	9.0	
J_{4a5e}	–	–	–	–	2	
J_{4a5a}	–	–	–	–	9	
J_{4e5a}	3.0	*ca.* 4	–		–	
J_{4e5e}	4.4	–	5.3		–	
J_{5a5e}	–13.6	–	–			
J_{5a6a}	11.7	12.0				
J_{5a6e}	4.8	*ca.* 2				
J_{5e6a}	3.2	–	5.4			
J_{5e6e}	3.4	–	3.4			
J_{6a6e}	–13.4	–13.0	–13.6			
J_{7a7e}	–	–	–	–14		

[a] The spectra of *6a,c,d*, *12*, and *13* were recorded in $CDCl_3$ and that of *19* in D_2O solutions. [b] Only the sum of J_{2a3a} and J_{2e3a} is precisely determined.

(*16*) by a modified von Braun procedure[9] followed by acid hydrolysis and dehydration of the intermediate *17* (Scheme 2) gave the rigid guvacine analogue *18*. Hydrogenation of *18* proceeded stereospecifically, *19* being the only detectable product.

The structure elucidation of the new products *2b – 4b*, *5 – 10*, *12 – 15*, and *17 – 19* were based on elemental analyses, IR and ^{1}H NMR spectroscopy, in the cases of *9a,b*, *10*, *14*, and *18* supported by UV spectroscopy. In tetrachloromethane solution the *β*-oxoester *4b* (Scheme 2) exists in the enol-form as established by ^{1}H NMR spectroscopy. The relative configurations of *5 – 8*, *12*, *13*, and *19* were established by analysis of the 270 MHz ^{1}H NMR spectra of the methyl esters *6a,c,d*, *12*, and *13* and compound *19*, respectively.

In simple piperidine derivatives the equatorial proton on C(2) is found downfield from its axial counterpart.[10] The coupling constants between the C(3) proton and the two C(2) protons are typical for equatorial-axial and axial-axial configurations of these protons in *6a*. This is consistent with a predominantly equatorial orientation of the C(3) methoxycarbonyl group (Table 1). Furthermore the coupling constant for the C(3) and C(4) protons unequivocally indicates axial-equatorial orientation of the protons concerned and therefore a 3,4-*cis* configuration of *6a*. An analysis of the mutual coupling constants for the C(4) and C(5) protons supports this assignment. The vicinal coupling constants used in this assignment are in general agreement with those found in most 6-membered rings.[11] The geminal coupling constants found parallel data previously found in other piperidines.[12] The coupling patterns of the C(2), C(3), and C(4) protons in *6c* are very similar to those of the corresponding system in *6a* establishing equatorial and axial positions of the substituents at C(3) and C(4), respectively. The coupling constants for the C(5) proton indicate an equatorial orientation of the C(5) methyl group. These findings together are in agreement with the depicted relative configuration of *6c*. In *6d* the mutual coupling constants for the C(6) and C(5) protons indicate an axial orientation of the C(5) methyl group. A further analysis of the C(5), C(4), and C(3) proton coupling patterns reveals axial and equatorial orientations of the hydroxy and C(3) methoxycarbonyl groups, respectively.

In previous studies of piperidines[10] it has been suggested that the chemical shift difference between axial and equatorial protons on C(2) and C(6) ($\Delta\delta$) would indicate the extent to which the nitrogen lone pair influences the two protons. The differences $\Delta\delta(H_{2a},H_{2e})$ and $\Delta\delta(H_{6a},H_{6e})$ found in *6a* would be consistent with the dominating contribution from the equatorial-axial orientation of the C(3) and C(4) substituents of *6a*. The $\Delta\delta$ values found for *6c* are larger, indicating an increased fixation in an equatorial-axial-equatorial arrangement of the C(3), C(4), and C(5) substituents. This tendency is reflected in the values of the axial-axial coupling constants, where $J_{2a,3a} = 12.0$ Hz would be consistent with an almost locked conformation. In *6d* the spectrum is quite different. The two protons at C(2) are almost coinciding in chemical shift at a value of *ca.* 3.7 ppm, the lack of accuracy arising from the near coincidence of the signal with those of the methoxy groups. The chemical shift difference between the two protons at C(2) can be judged from the triplet structure of the C(3) proton in the ABX pattern formed by the three protons at C(2) and C(3). The vanishingly small chemical shift difference on C(2) and the reduced chemical shift difference on C(6) as well as the smaller value of $J_{3a,4a}$ in *6d* (10.0 Hz) all point to a conformational equilibrium where equatorial-axial-axial orientations of the C(3), C(4), and C(5) substituents represent the preferred but not the exclusive conformation.

The 270 MHz ^{1}H NMR spectra of *12* and *13* were analysed as far as the complexity of the spectra permitted. Interpretation of the spectra, recorded at 293 K, was complicated by the fact that both molecules gave rise to two sets of signals due to hindered rotation of the urethane group. This is reflected in dual values for several chemical shifts while the coupling constants determined in both conformers were identical. The coupling constants clearly indicate that the arrangement of the substituents in position 3 and 4 are equatorial-axial in *12* and equatorial-equatorial in *13*. The observed values for chemical shifts and coupling constants are in general agreement with the data given for *6a*. In the spectrum of *19* a merging and very complex pattern of signals from the C(3), C(4), C(6), and C(7) protons is observed. It is not possible to establish the orientation of the carboxyl group of *19* on the basis of the coupling constant for the equatorial C(1) proton and the proton on C(2). However, the total width of the pattern originating in the C(2) proton reveals the existence of an axial-axial coupling between this proton and the axially oriented C(3) proton thus indicating an equatorial orientation of the carboxyl group.

EXPERIMENTAL

Unless otherwise stated, the determination of melting points and elemental analyses and the recording of IR, UV, and 60 MHz ^{1}H NMR spectra were accomplished as described in a previous paper.[13] ^{1}H NMR spectra of compounds dissolved in D_2O were recorded by using sodium 3-(trimethylsilyl)propanesulfonate as an in-

ternal standard. The 270 MHz ^{1}H NMR spectra were obtained on a Bruker HX 270 S instrument operating at 293 and 303 K. Fourier transform method was used to obtain the spectra with a spectral width of 1500 Hz using 32 K data points. The detection was quadrature detection. Homodecoupling was used to verify the interpretations of the spectra and provide starting parameters for the analyses of the spectra. Nitrogen decoupling was possible using a Bruker probehead equipped with an additional nitrogen decoupling coil. The frequency was derived from a Bruker synthesizer model BS 100 and fed to the amplifier of the decoupling channel through a band pass filter before entering the probe. The decoupling power was 5 watt. The decoupling frequency used was 19 506 582 Hz. The proton spectra were analyzed using the program SIMEQ and a Varian 620/i computer. Due to the large chemical shift differences at 270 MHz, it is permissible to subdivide the total spin system into several subsystems containing typically 3 to 6 nuclei. After the initial assignment the spectra were iterated to a best fit using the ITRCAL program on a Nicolet 1180 computer with 80 K memory. Optical rotations were measured on a Perkin-Elmer polarimeter model 141. Thin-layer chromatography (TLC) and column chromatography (CC) were accomplished by using silica gel F_{254} plates (Merck) and silica gel (Woelm 0.063–0.1 mm), respectively. Columns were developed by stepwise gradient elution. The pK_A values were determined as described in a previous paper.[14]

(RS)-Ethyl N-benzyl-N-(2-ethoxycarbonylethyl)-2-methyl-3-aminopropionate (2b). To a solution of *1* (107 g; 1 mol) in ethanol (200 ml) was added ethyl methacrylate (137 g; 1.2 mol). The mixture was refluxed for 24 h, the solvent removed *in vacuo*, and the residue distilled to give (*RS*)-ethyl *N*-benzyl-2-methyl-3-aminopropionate (149.9 g; 68 %), b.p. 131–133 °C/80 Pa. Anal. $C_{13}H_{19}NO_2$: C, H, N. IR (film): 3330 (w), 3030 (w), 2970–2820 (m), 1725 (s), 1495 (m), 1450(m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 7.17 (5 H, s), 3.98 (2 H, q, *J* 7 Hz), 3.63 (2 H, s), 3.0–2.1 (3 H, m), 1.42 (1 H, s), 1.33–0.97 (6 H, m).

A solution of (*RS*)-ethyl *N*-benzyl-2-methyl-3-aminopropionate (142.3 g; 0.65 mol) and ethyl acrylate (83.9 g; 0.84 mol) in ethanol (150 ml) was refluxed for 4 days. The solvent was removed *in vacuo*, and distillation of the residue gave *2b* (164.8 g; 79 %), b.p. 165–167 °C/47 Pa. Anal. $C_{18}H_{27}NO_4$: C, H, N. IR (film): 3030 (w), 2980 (m), 2810 (m), 1730 (s), 1495 (w), 1455 (m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 7.18 (5 H, s), 4.2–3.7 (4 H, dq), 3.52 (2 H, s), 2.9–2.0 (7 H, m), 1.4–0.9 (9 H, dt+m).

(RS)-Ethyl N-methoxycarbonyl-N-(2-ethoxycarbonylethyl)-2-methyl-3-aminopropionate (3b). A solution of *2b* (79.95 g; 250 mmol) and aqueous hydrochloric acid (62.5 ml; 4 M) in ethanol (200 ml) was hydrogenated (*ca.* 250 kPa) in a PARR hydrogenation apparatus by using a 10 % Pd-C catalyst (5.6 g). The reaction mixture was filtered and evaporated *in vacuo*. To an ice-cooled solution of the residue in water (100 ml) was added with stirring an iced solution of potassium carbonate (86.94 g; 630 mmol) in water (100 ml) followed by addition of methyl chloroformate (28.20 g; 300 mmol). Stirring was continued at 0 °C for 30 min and at 25 °C for 1 h. The mixture was extracted with ether (3 × 200 ml). The combined and dried (K_2CO_3) ether phases were evaporated *in vacuo* and the residue distilled to give *3b* (46.4 g; 64 %), b.p. 143–145 °C/53 Pa. Anal. $C_{13}H_{23}NO_4$: C, H, N. IR (film): 2980 (m), 1730 (s), 1705 (s), 1485 (m), 1445 (m), 1410 (m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 4.02 (4 H, q, *J* 7 Hz), 3.59 (3 H, s) 3.5–3.1 (4 H, m), 3.0–2.0 (3 H, m), 1.4–0.8 (9 H, m).

(RS)-Ethyl 1-methoxycarbonyl-4-hydroxy-5-methyl-1,2,5,6-tetrahydropyridine-3-carboxylate (4b). To a suspension of sodium (1.84 g; 80 mmol) in benzene-xylene (10:1) (80 ml) was added ethanol (9.2 g; 200 mmol). When the sodium was dissolved a solution of *3b* (23.2 g; 80 mmol) in benzene (40 ml) was added with stirring. The reaction mixture was left for 3 days at 25 °C. Upon addition of hydrochloric acid (25 ml; 4 M) the organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to give crude *4b* (14.3 g). CC [silica gel: 400 g; eluents: benzene containing ethyl acetate (5–15 %)] followed by distillation gave *4b* (8.1 g; 42 %), b.p. 124–128 °C/40 Pa. Anal. $C_{11}H_{17}NO_5$: C, H, N. IR (film): 2980 (w), 2870 (w), 1710 (s), 1655 (m), 1620 (m), 1450 (m), 1410 (m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 12.17 (1 H, s), 4.6–3.8 (4 H, m), 3.8–3.0 (5 H, m), 2.7–2.1 (1 H, m), 1.6–0.7 (6 H, m).

(3RS,4SR)-Ethyl 1-methoxycarbonyl-4-hydroxypiperidine-3-carboxylate (5a). A solution of *4a* [15] (55.0 g; 0.24 mol) in ethanol (500 ml) was hydrogenated (*ca.* 10 MPa) by using a Ra-Ni W-2 catalyst (9 g). The filtered and evaporated reaction product was distilled to give *5a* (50.0 g; 90 %), b.p. 145–149 °C/9 Pa, m.p. 51.5–53.5 °C. Anal. $C_{10}H_{17}NO_5$: C, H, N. IR (film): 3600–3200 (m), 2990–2850 (several bands, m), 1725 (s), 1705 (s) cm^{-1}.

(3RS,4SR)-Dimethyl 4-hydroxypiperidine-1,3-dicarboxylate (6a). A solution of *5a* (26 g; 0.11 mol) in a methanolic solution of hydrogen chloride (500 ml; 5 %) was refluxed for 18 h. The evaporated reaction mixture was distilled to give *6a* (21.0 g; 86 %), b.p. 143–146 °C/9 Pa, m.p. 68.5–70.5 °C. Anal. $C_9H_{15}NO_5$: C, H, N. IR (KBr): 3450 (s), 3000–2830 (several bands, m), 1720 (s), 1685 (s), 1670 (s) cm^{-1}.

(3RS,4SR)-3-Carboxy-4-hydroxypiperidinium chloride (7a). A solution of *6a* (4.0 g; 18.4 mmol) in hydrochloric acid (40 ml; 5 M) was refluxed for 150 min. The evaporated oily reaction product was crystallized from water-glacial acetic acid [35 ml; 2:5)] to give *7a* (1.6 g;

48 %), m.p. 170.0 – 172.0 °C. Anal. $C_5H_{12}ClNO_3$: C, H, Cl, N. IR (KBr): 3600 – 3350 (s), 3160 – 2740 (several bands, s), 2720 – 2400 (several bands, m), 1720 (s), 1600 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 4.6 – 4.4 (1 H, m), 3.5 – 2.8 (5 H, m), 2.2 – 1.8 (2 H, m).

(3RS,4SR)-4-Hydroxypiperidine-3-carboxylic acid zwitterion (8a). To a solution of *7a* (1.32 g; 10 mmol) in water (5 ml) was added a solution of triethylamine (1.06 g; 10.5 mmol) in ethanol (5 ml). To the filtered solution was added *N,N*-dimethylformamide (4 ml). Upon standing at 25 °C for 7 days *8a* (890 mg; 61 %) was isolated, m.p. 253 – 255 °C (decomp.). Anal. $C_6H_{11}NO_3$: C, H, N. IR (KBr): 3210 (s), 3100 – 2200 (several bands, s), 1615 (s), 1550 (s), 1540 (s) cm^{-1}. pK_A values (H_2O, 22 °C): 3.42 ± 0.05; 10.02 ± 0.05.

(3RS,4SR,5SR)-Ethyl 1-methoxycarbonyl-4-hydroxy-5-methylpiperidine-3-carboxylate (5c) and (3RS,4SR,5RS)-ethyl 1-methoxycarbonyl-4-hydroxy-5-methylpiperidine-3-carboxylate (5d). A solution of *4b* (4.0 g; 16 mmol) in ethanol (130 ml) was hydrogenated (*ca.* 3 MPa) by using an Ra-Ni W-2 catalyst (3 g) for 23 h. The filtered and evaporated reaction mixture was shown by TLC [eluent: benzene-ethyl acetate (4:1)] to consist of two compounds (R_F = 0.24 and 0.17). CC [silica gel: 200 g; eluents: benzene containing ethyl acetate (15 – 25 %)] of the crude product and ball-tube distillation of the separated components at 133 Pa (oven temperature 180 °C) gave pure *5c* and *5d*. *5c* (1.45 g; 37 %) had m.p. 79.0 – 79.5 °C. Anal. $C_{11}H_{19}NO_5$: C, H, N. IR (KBr): 3470 (m), 2950 (m), 2900 (m), 1725 (s), 1685 (s), 1480 (s), 1455 (m), 1440 (m), 1415 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.4 – 3.9 (m) and 3.67 (s) (a total of 7 H), 3.4 – 2.0 (5 H, m), 1.9 – 0.8 (7 H, m). *5d* (1.12 g; 29 %) had m.p. 45 – 46 °C. Anal $C_{11}H_{19}NO_5$: C, H, N. IR (film): 3470 (m), 2955 (m), 2925 (m), 1730 (s), 1700 (s), 1685 (s), 1480 (m), 1445 (m), 1415 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.10 (q, *J* 7 Hz) and 3.9 – 2.2 (m) (a total of 12 H), 2.2 – 1.5 (1 H, m), 1.5 – 0.7 (6 H, m).

(3RS,4SR,5SR)-Dimethyl 4-hydroxy-5-methylpiperidine-1,3-dicarboxylate (6c). A solution of *5c* (531 mg; 2.2 mmol) in methanolic hydrogen chloride (10 ml; 5 %) was refluxed for 17 h. Ball-tube distillation of the evaporated reaction product at 133 Pa (oven temperature 180 °C) gave *6c* (472 mg; 93 %), m.p. 80.0 – 81.0 °C. The IR spectrum was almost identical with that of *5c*.

(3RS,4SR,5SR)-3-Carboxy-4-hydroxy-5-methylpiperidinium chloride (7c). A solution of *6c* (1.25 g; 5.4 mmol) in aqueous hydrochloric acid (50 ml; 5 M) was refluxed for 75 min. Evaporation of the reaction mixture *in vacuo* and recrystallization (water – acetic acid) of the residue gave *7c* (486 mg; 46 %), m.p. 232.5 – 233.5 °C. Anal. $C_7H_{14}ClNO_3$: C, H, Cl, N. IR (KBr): 3400 (m), 3145 (m), 2940 (m), 2800 (m), 1720 (s), 1605 (m), 1465 (m), 1450 (m), 1420 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 4.23 (1 H, slightly broadened s), 3.6 – 2.5 (5 H, m), 2.3 – 1.6 (1 H, m), 1.00 (3 H, d, *J* 7 Hz).

(3RS,4SR,5SR)-4-Hydroxy-5-methylpiperidine-3-carboxylic acid zwitterion (8c). To a solution of *7c* (100 mg; 0.51 mmol) in water (1 ml) was added a solution of triethylamine (57 mg; 0.56 mmol) in ethanol (1 ml). The mixture was left at 4 °C for 17 h. Recrystallization (water – ethanol) of crude *8c* gave pure *8c* (46 mg; 51 %), m.p. 116.0 – 117.0 °C. Anal. $C_7H_{13}NO_3.H_2O$: C, H, N. IR (KBr): 3700 – 3100 (s), 3100 – 2400 (several bands, m), 1590 (s), 1470 (m), 1395 (s) cm^{-1}. pK_A values (H_2O, 23 °C): 3.39 ± 0.02; 10.13 ± 0.04.

(3RS,4SR,5RS)-Dimethyl 4-hydroxy-5-methylpiperidine-1,3-dicarboxylate (6d). *6d* was synthesized as described above for *6c* by using *5d* (471 mg; 1.9 mmol) as a starting material. Purification of the crude product by ball-tube distillation at 133 Pa (oven temperature 180 °C) gave *6d* (404 mg; 92 %). The IR spectrum was almost identical with that of *5d*.

(3RS,4SR,5RS)-3-Carboxy-4-hydroxy-5-methylpiperidinium chloride (7d). *7d* was synthesized as described above for *7c* by using *6d* (1.25 g; 5.4 mmol) as a starting material. Recrystallization (water – acetic acid) of the crude product gave *7d* (297 mg; 28 %), m.p. 211.5 – 212.0 °C. Anal. $C_7H_{14}ClNO_3$: C, H, Cl, N. IR (KBr): 3445 (m), 3110 (m), 3000 (m), 2845 (m), 1720 (s), 1585 (m), 1470 (m), 1435 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 4.0 – 3.7 (1 H, m), 3.7 – 2.6 (5 H, m), 2.4 – 1.8 (1 H, m), 1.07 (3 H, d, *J* 7 Hz).

(3RS,4SR,5RS)-4-Hydroxy-5-methylpiperidine-3-carboxylic acid zwitterion (8d). *8d* was synthesized as described above for *8c* by using *7d* (100 mg; 0.51 mmol) as a starting material. Recrystallization (water-ethanol) of the crude product gave *8d* (28 mg; 31 %), m.p. 234.0 – 235.0 °C. Anal. $C_7H_{13}NO_3.H_2O$: C, H, N. IR (KBr): 3600 – 3350 (m), 3350 – 2800 (several bands, s – m), 1730 (m), 1600 (s), 1470 (m), 1410 (s) cm^{-1}. pK_A values (H_2O, 23 °C): 3.28 ± 0.03; 9.99 ± 0.06.

Guvacine hydrobromide (3-carboxy-1,2,5,6-tetrahydropyridinium bromide) (9a). A solution of *6a* (13.5 g; 62 mmol) in aqueous hydrobromic acid (60 ml; 48 %) was refluxed for 24 h. Upon cooling of the reaction mixture to 5 °C pure *9a* (10.4 g; 30 %) crystallized, m.p. 280 °C (decomp.). Anal. $C_6H_{10}BrNO_2$: C, H, Br, N. IR (KBr): 3175 (m), 2950 (s), 2820 (m), 2620 – 2400 (several bands, w), 1720 (s), 1660 (m), 1580 (m) cm^{-1}. UV [methanol (log ε)]: 212 (4.00) nm. ^1H NMR (60 MHz, D_2O): δ 7.3 – 7.0 (1 H, m), 3.9 – 3.7 (2 H, q), 3.5 – 3.1 (2 H, t), 2.7 – 2.3 (2 H, m). pK_A values (H_2O, 22 °C): 3.50 ± 0.06; 9.85 ± 0.06.

(RS)-3-Carboxy-5-methyl-1,2,5,6-tetrahydropyridinium bromide (9b). A solution of *6c* (517 mg; 1.99 mmol) in aqueous hydrobromic acid (1.5 ml; 48 %) was refluxed for 24 h. Upon cooling of the reaction mixture to 24 °C TLC-pure *9b* crystallized (162 mg; 37 %) [R_F:

0.22; eluent: 1-butanol – acetic acid – water (4:1:1)]. An analytical sample was recrystallized (water – acetic acid) to give *9b*, m.p. 253.5 – 254.5 °C. Anal. $C_7H_{12}BrNO_2$: C, H, Br, N. IR (KBr): 3550 – 3300 (m), 3250 – 2500 (several bands, s – m), 1710 (s), 1660 (m), 1605 (m), 1400 (m) cm^{-1}. UV [methanol (log ε)]: 214 (4.00) nm. ^1H NMR (60 MHz, D_2O): δ 7.2 – 6.9 (1 H, m), 3.9 – 3.7 (2 H, broadened s), 3.7 – 3.2 (1 H, m), 3.1 – 2.5 (2 H, m), 1.17 (3 H, d, J 7 Hz).

(RS)-5-Methyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid zwitterion (10). *10* was synthesized as described above for *3c* by using *9b* (100 mg; 0.56 mmol) and a solution of triethylamine (63 mg; 0.62 mmol) in ethanol (2 ml). Recrystallization (water – ethanol) of the crude product gave *10* (37 mg; 47 %), m.p. 249.0 – 249.5 °C. Anal. $C_7H_{11}NO_2$: C, H, N. IR (KBr): 3600 – 3250 (m), 3200 – 2000 (several bands, m), 1660 (s), 1630 (s), 1550 (s), 1470 (m) cm^{-1}. UV [methanol (log ε)]: 210 (3.38) nm. pK_A values (H_2O, 23 °C): 3.29 ± 0.03; 9.35 ± 0.03.

(3RS,4SR)-Dimethyl 4-hydroxyperhydroazepine-1,3-dicarboxylate (12) and (3RS,4RS)-dimethyl 4-hydroxyperhydroazepine-1,3-dicarboxylate (13). A mixture of *12* and *13* was prepared from *11* [16] (2.56 g; 10.5 mmol) in ethanol (15 ml) by using 1.5 g of catalyst as described above for *5c,d*, followed by reflux of a solution of the crude reduction product in methanolic hydrogen chloride (150 ml; 15 %) for 24 h. CC [silica gel: 180 g; eluents: benzene containing ethyl acetate (15 – 25 %)] of the evaporated reaction product followed by ball-tube distillation at 60 Pa (oven temperature 180 °C) of the isolated components gave *12* (1.68 g; 69 %), m.p. 55.5 – 57.5 °C. Anal. $C_{10}H_{17}NO_5$: C, H, N. IR (KBr): 3450 (m), 2950 (m), 2930 (m), 1735 (s), 1705 – 1675 (s), 1485 – 1440 (m), 1410 (m) cm^{-1}. 349 mg (14 %) of *13* was obtained. Anal. $C_{10}H_{17}NO_5$: C, H, N. IR (film): 3450 (m), 2950 (m), 2930 (m), 1735 (s), 1705 – 1680 (s), 1485 – 1440 (m), 1410 (m) cm^{-1}.

3-Carboxy-2,5,6,7-tetrahydro-1H-azepinium bromide (14). A solution of *12* (370 mg; 1.6 mmol) in hydrobromic acid (1 ml; 48 %) was refluxed for 24 h. Evaporation *in vacuo* and recrystallization (water – isopropanol) of the residue gave *14* (225 mg; 63 %), m.p. 224.5 – 225.5 °C. Anal. $C_7H_{12}BrNO_2$: C, H, Br, N. IR (KBr): 3430 (m), 3150 – 2700 (several bands, s – m), 1715 (s), 1650 (m), 1575 (m), 1460 (m), 1420 (m) cm^{-1}. UV [methanol (log ε)]: 210 (4.00) nm. ^1H NMR (60 MHz, D_2O): δ 7.55 (1 H, t, J 7 Hz), 4.12 (2 H, s), 3.6 – 3.3 (2 H, m), 2.8 – 2.3 (2 H, m), 2.2 – 1.6 (2 H, m). pK_A values (H_2O, 23 °C): 3.71 ± 0.05; 10.20 ± 0.03.

(RS)-3-Carboxyperhydroazepinium bromide (15). A solution of *14* (490 mg; 2.2 mmol) in aqueous ethanol (150 ml; 35 %) was hydrogenated (*ca.* 300 kPa) in a PARR hydrogenation apparatus by using a 10 % Pd – C catalyst (100 mg). Evaporation *in vacuo* and recrystallization of the residue (water – acetic acid) gave *15* (368 mg; 74 %), m.p. 145.0 – 146.5 °C. Anal. $C_7H_{14}BrNO_2$: C, H, Br, N. IR (KBr): 3700 – 3300 (m), 3250 – 2900 (s), 2860 (m), 2750 – 2400 (several bands, m – w), 1730 (s), 1580 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 3.7 – 2.9 (5 H, m), 2.4 – 1.6 (6 H, m). pK_A values (H_2O, 23 °C): 3.33 ± 0.04; 10.58 ± 0.04.

(1R,2R,3S,5S)-(–)-Methyl 3-ethoxycarbonyloxy-8-ethoxycarbonylnortropane-2-carboxylate (17). A solution of ecgonine hydrate (*16*) (3.0 g; 14.8 mmol) in methanolic hydrogen chloride (200 ml; 5 %) was refluxed for 4 h. Upon evaporation *in vacuo* an aqueous solution (7 ml) of the residue, adjusted to pH 10 by sodium hydroxide, was extracted with chloroform (3 × 30 ml). The combined, dried (Na_2CO_3), and evaporated organic phases were submitted to ball-tube distillation at 130 Pa (oven temperature 150 °C) to give ecgonine methyl ester (2.5 g; 85 %) as a TLC-pure oil [R_F: 0.11; eluent: 1-butanol – acetic acid – water (4:1:1)]. A solution of ecgonine methyl ester (2.5 g; 12.6 mmol) and ethyl chloroformate (14.0 g; 130 mmol) in 1,2-dichloroethane (70 ml) was refluxed for 3 days. Evaporation *in vacuo* and CC [silica gel: 180 g; eluents: dichloromethane containing ethyl acetate (20 – 50 %) and methanol (3 %)] of the oily residue followed by ball-tube distillation at 100 Pa (oven temperature 170 °C) gave *17* (1.63 g; 39 %), m.p. 92.0 – 93.5 °C. Anal. $C_{13}H_{23}NO_7$: C, H, N. $[\alpha]_D^{25}$ – 48° (*c* 0.91, ethanol). IR (KBr): 3600 – 3100 (m), 2980 (m), 2890 (w), 1735 (s), 1710 (s), 1480 (m), 1435 (s) cm^{-1}. ^1H NMR (60 MHz, CCl_4): δ 5.2 – 4.7 (1 H, m), 4.7 – 4.3 (2 H, m), 4.3 – 3.8 (4 H, dq, each J 7 Hz), 3.66 (3 H, s), 3.1 – 2.3 (2 H, q), 2.7 – 2.2 (1 H, m), 2.0 – 1.6 (4 H, m), 1.5 – 1.2 (6 H, dt, each J 7 Hz).

(1R,5S)-(–)-2-Nortropene-2-carboxylic acid hydrobromide (18). A solution of *17* (825 mg; 2.5 mmol) in aqueous hydrobromic acid (6 ml; 48 %) was refluxed for 5 h. Evaporation *in vacuo* and recrystallization (2-propanol – ether) of the residue gave *18* (389 mg; 66 %), m.p. 278.5 – 280.5 °C. Anal. $C_8H_{12}BrNO_2$: C, H, Br, N. $[\alpha]_D^{25}$ – 61° (*c* 0.98, water). IR (KBr): 3080 (s), 2890 (s), 2730 – 2440 (several bands, m – w), 1690 (s), 1640 (m), 1600 (m) cm^{-1}. UV [methanol (log ε)]: 212 (3.95) nm. ^1H NMR (60 MHz, D_2O): δ 7.2 – 7.0 (1 H, t, J 4 Hz), 4.8 – 4.6 (1 H, m), 4.5 – 4.2 (1 H, m), 3.4 – 2.4 (2 H, m), 2.4 – 1.9 (4 H, m). pK_A values (H_2O, 23 °C): 3.33 ± 0.03; 10.23 ± 0.05.

(1R,2R,5R)-(+)-Nortropane-2-carboxylic acid hydrobromide (19). *19* was prepared as described above for *15* from *18* (250 mg; 1.1 mmol) by using 50 mg of the catalyst. 203 mg (81 %) of *19* was obtained, m.p. 237.5 – 238.5 °C. Anal. $C_8H_{14}BrNO_2$: C, H, Br, N. $[\alpha]_D^{25}$ + 12° (*c* 0.98, water). IR (KBr): 3600 – 3300 (m), 3120 (m), 3000 – 2830 (s), 2800 – 2400 (several bands, m – w), 1730 (s), 1610 (m) cm^{-1}.

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REFERENCES

1. Krogsgaard-Larsen, P. and Johnston, G. A. R. *J. Neurochem. 25* (1975) 797.
2. Johnston, G. A. R., Krogsgaard-Larsen, P., Stephanson, A. L. and Twitchin, B. *J. Neurochem. 26* (1976) 1029.
3. Johnston, G. A. R., Stephanson, A. L. and Twitchin, B. *J. Neurochem. 26* (1976) 83.
4. Johnston, G. A. R., Krogsgaard-Larsen, P. and Stephanson, A. L. *Nature 258* (1975) 627.
5. Krogsgaard-Larsen, P. In Fonnum, F., Ed., *Amino Acids as Neurotransmitters*, Plenum, New York 1978. *In press.*
6. McElvain, S. M. and Stork, G. *J. Am. Chem. Soc. 68* (1946) 1049.
7. Morlacchi, F., Cardellini, M. and Liberatore, F. *Ann. Chim. Rome 57* (1967) 1456.
8. Johnston, G. A. R. *To be published.*
9. Fischer, J. G. and Mikite, G. *Acta Chim. Acad. Sci. Hung. 68* (1971) 253.
10. Lambert, J. B. and Keske, R. G. *J. Am. Chem. Soc. 88* (1966) 620.
11. Jackman, L. M. and Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon, Oxford 1969, p. 288.
12. Lambert, J. B., Keske, R. G., Carhart, R. E. and Jovanovich, A. P. *J. Am. Chem. Soc. 89* (1967) 3761.
13. Krogsgaard-Larsen, P., Christensen, S. B. and Hjeds, H. *Acta Chem. Scand. 27* (1973) 2802.
14. Brehm, L., Krogsgaard-Larsen, P. and Hjeds, H. *Acta Chem. Scand. B 28* (1974) 308.
15. Krogsgaard-Larsen, P. and Hjeds, H. *Acta Chem. Scand. B 28* (1974) 533.
16. Krogsgaard-Larsen, P. and Hjeds, H. *Acta Chem. Scand. B 30* (1976) 884.

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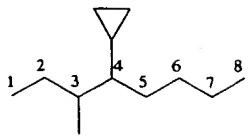

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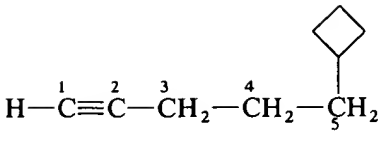

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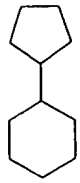
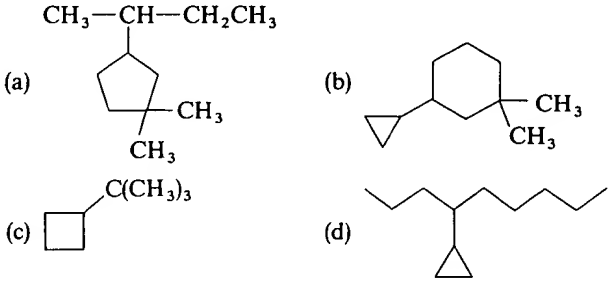


cyclopentylcyclohexane

PROBLEM 3-14

Give a IUPAC name for each of the following compounds.



PROBLEM 3-15

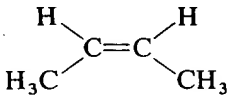
Draw the structure and give the molecular formula for each of the following compounds.

(a) cyclododecane
(b) propylcyclohexane
(c) cyclopropyl cyclopentane
(d) 3-ethyl-1,1-dimethylcyclohexane

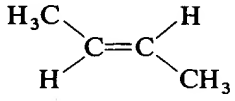
3-6D GEOMETRIC ISOMERISM IN CYCLOALKANES

Open-chain alkanes undergo rotations about their carbon-carbon single bonds, and they are free to assume any of an infinite number of conformations. Alkenes have rigid double bonds that prevent rotation, giving rise to cis and trans isomers with different orientations of the groups on the double bond. Cycloalkanes are similar to alkenes in this respect. A cycloalkane has two distinct faces. If two substituents point toward the same face, they are cis. If they point toward opposite faces, they are trans. These geometric isomers cannot interconvert without breaking and re-forming bonds.

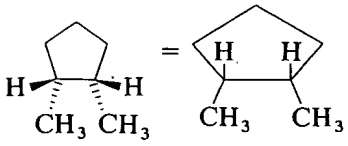
Figure 3-15 compares the geometric isomers of 2-butene with those of 1,2-dimethylcyclopentane. You should make models of these compounds to convince



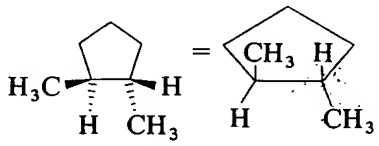
cis-2-butene



trans-2-butene



cis-1,2-dimethylcyclopentane



trans-1,2-dimethylcyclopentane

FIGURE 3-15 Like alkenes, cycloalkane rings are restricted from free rotation. Two substituents on a cycloalkane must be either on the same side (cis) or on opposite sides (trans) of the ring.

Both of these conformations require one group to be axial while the other is equatorial. The ethyl group is bulkier than the methyl group, so the conformation with the ethyl group equatorial is more stable. These chair conformations are in equilibrium at room temperature, and the one with the equatorial ethyl group predominates.

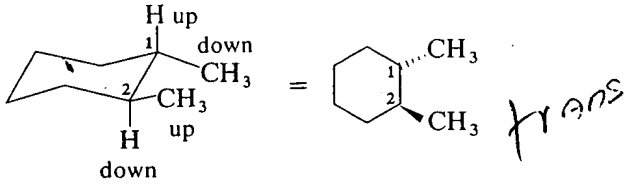
PROBLEM 3-26

Draw the two chair conformations of each of the following substituted cyclohexanes. In each case, label the more stable conformation.

(a) *cis*-1-ethyl-2-methylcyclohexane
(b) *trans*-1-ethyl-2-methylcyclohexane
(c) *cis*-1-ethyl-4-isopropylcyclohexane
(d) *trans*-1-ethyl-4-methylcyclohexane

Recognizing cis and trans isomers

Some students find it difficult to look at a chair conformation and tell whether a disubstituted cyclohexane is the cis isomer or the trans isomer. In the following drawing the two methyl groups appear to be oriented in similar directions. They are actually trans but are sometimes mistakenly identified as cis.



trans-1,2-dimethylcyclohexane

This ambiguity is eliminated by recognizing that each of the ring carbon atoms has two available bonds, one directed upward and one directed downward. In this drawing the methyl group on C1 is on the downward bond, and the methyl on C2 is on the upward bond. Because one is on a downward bond and one on an upward bond, their relationship is trans. A cis relationship would require both groups to be upward or both to be downward.

PROBLEM 3-27

Name each of the following compounds, remembering that two up bonds are cis; two down bonds are cis; one up bond and one down bond are trans.